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| FORM PTO-1390 (Modified) (REV 11-2000) | | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | | ATTORNEY'S DOCKET NUMBER 24900-501NATL | |
| TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371 | | | | U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) 10/069210 | |
| INTERNATIONAL APPLICATION NO. PCT/CA00/00974 | | INTERNATIONAL FILING DATE 22 August 2000 (22.08.2000) | | PRIORITY DATE CLAIMED 23 August 1999 (23.08.1999) | |
| TITLE OF INVENTION RADIOACTIVELY COATED DEVICE AND METHOD OF MAKING SAME FOR PREVENTING RESTENOSIS | | | | | |
| APPLICANT(S) FOR DO/EO/US LECLERC, Guy; FAREH, Jeanette; LEBLANC, Phillippe; LEVESQUE, Luc; MARTEL, Remi; KUDREVICH, Svetlana; LAWRENCE, Marcus F.; BOURGUIGNON, Bernard; LESSARD, Jean; (cont. under item 23) | | | | | |
| Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: | | | | | |
| <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below. 4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). | | | | | |
| Items 13 to 20 below concern document(s) or information included: | | | | | |
| <ol style="list-style-type: none"> 13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 17. <input type="checkbox"/> A substitute specification. 18. <input type="checkbox"/> A change of power of attorney and/or address letter. 19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail 23. <input checked="" type="checkbox"/> Other items or information: | | | | | |
| Additional Applicants: BLAIS, Sonia; CHAPUZET, Jean-Marc; MEUNIER, Michel; NAPPORN, Teko; POULIN, Suzie; SACHER, Edward; SAVADOGO, Oumarou. Express Mail Label No. EL 862123240 US; Filed on 22 February 2002 (22.02.02) Form PCT/IB/308 enclosed as proof of filing in United States Receiving Office | | | | | |

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|---|--------------|--|--------------|--|----|
| U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <div style="font-size: 2em; font-weight: bold; text-align: center;">10/069210</div> | | INTERNATIONAL APPLICATION NO. <div style="text-align: center;">PCT/CA00/00974</div> | | ATTORNEY'S DOCKET NUMBER <div style="text-align: center;">24900-501NATL</div> | |
| 24. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : <div style="margin-left: 20px;"><input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00</div> <div style="text-align: right; margin-right: 50px;">ENTER APPROPRIATE BASIC FEE AMOUNT = \$890.00</div> | | | | CALCULATIONS PTO USE ONLY | |
| Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 | | | | \$130.00 | |
| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
| Total claims | 49 - 20 = | 29 | x \$18.00 | \$522.00 | |
| Independent claims | 6 - 3 = | 3 | x \$84.00 | \$252.00 | |
| Multiple Dependent Claims (check if applicable). <input type="checkbox"/> | | | | \$0.00 | |
| TOTAL OF ABOVE CALCULATIONS = | | | | \$1,794.00 | |
| <input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2. | | | | \$897.00 | |
| SUBTOTAL = | | | | \$897.00 | |
| Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30 + | | | | \$0.00 | |
| TOTAL NATIONAL FEE = | | | | \$897.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/> | | | | \$0.00 | |
| TOTAL FEES ENCLOSED = | | | | \$897.00 | |
| | | | | Amount to be: refunded | \$ |
| | | | | charged | \$ |
| a. <input checked="" type="checkbox"/> A check in the amount of <u> \$897.00 </u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u> 50-0311 </u> A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. | | | | | |
| NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. | | | | | |
| SEND ALL CORRESPONDENCE TO: | | | | | |
| <div style="border: 1px solid black; padding: 5px;">ELRIFI, Ivor R. Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C. One Financial Center Boston, Massachusetts 02111 United States of America</div> | | | | | |
| <div style="margin-bottom: 10px;"> SIGNATURE</div> <div style="margin-bottom: 10px;">MORENCY, Michel NAME</div> <div style="margin-bottom: 10px;">50,183 REGISTRATION NUMBER</div> <div style="margin-bottom: 10px;">22 February 2002 (22.02.02) DATE</div> | | | | | |

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Attorney Docket No. 24900-501 NATL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Leclerc, *et al.*
ASSIGNEE: Angiogene Inc.
SERIAL NUMBER: To Be Assigned
FILING DATE: February 22, 2002
FOR: RADIOACTIVELY-COATED DEVICE AND METHOD OF MAKING SAME FOR
PREVENTING RESTENOSIS

EXAMINER: To Be Assigned
ART UNIT: To Be Assigned

February 22, 2002
Boston, Massachusetts

Box PCT
Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to calculation of filing fees for the present application and prior to action on the merits, Applicants respectfully request the following amendment be entered in the above-referenced application.

Applicants believe that no fees are due with the filing of this Preliminary Amendment. However, the Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment, to Deposit Account No. 50-0311, Reference No. 24900-501.

In the Specification:

At page 1, line 4, please insert the following:

-- RELATED APPLICATIONS

The present application claims priority from U.S.S.N. 60/149,897, filed August 23, 1999, and International Application Number PCT/CA00/00974, filed August 22, 2000, each incorporated herein by reference in their entirety. --

In the Claims:

Please cancel claim 50.

APPLICANTS: Leclerc, *et al.*
U.S.S.N.: To Be Assigned

REMARKS

Upon entry of the present amendment, claims 1-49 are pending. Claim 50 is cancelled by the present amendment without prejudice or disclaimer. Applicant reserves the option to further prosecute the same or similar claims in this or in another patent application. No new matter has been added by the present amendment.

CONCLUSION

Applicants submit that the pending claims are in condition for allowance. If there are any questions regarding this amendment and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



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Dated: February 22, 2002

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WO 01/14617

PCT/CA00/00974

Radioactively coated device and method of making same
for preventing restenosis

BACKGROUND OF THE INVENTION

5 (a) Field of the Invention

The invention relates to a radioactively coated device and to a method of making same by deposition of a radioisotope-containing molecule on the device.

(b) Description of Prior Art

10 Although coronary angioplasty procedure reduces anginal symptoms, a high incidence of restenosis (30 to 40% within 6 months) is the "Achilles' heel" of interventional cardiology. With over one million coronary procedures performed annually around the
15 world, the economic effect of restenosis is substantial. It is estimated that an effective strategy to prevent restenosis, which would have to be applied after all coronary procedures, would represent a market of at least one billion U.S. dollars (US\$)
20 per year. Pharmacological approaches to prevent restenosis have failed to be effective and only coronary stenting procedure reduced restenosis rates (STRESS and BENESTENT trials). Stent deployment, however, frequently induces a new coronary occlusion
25 known as in-stent restenosis. About 20% of stented patients develop in-stent restenosis. To prevent occurrence of stenosis, new therapeutic strategies on the basis of ionizing radiation have recently been proposed. Intracoronary radiation therapy was
30 reported to prevent intimal hyperplasia in various animal models (Raizner et al., Chap 3: 287-296,

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Vascular Brachytherapy, Second Edition. Armonk, NY, 1999). In clinical development, endovascular radiotherapy (wire- and stent-based) in patients was reported to be safe and effective in preventing
5 restenosis post-angioplasty (Condado et al., *Circulation*, 90(3):727-732, 1997; Teirstein et al., *N. Engl. J. Med.*, 336(24):1697-1703, 1997; King et al., *Circulation*, 97:2025-2030, 1998; Waksman et al., *Circulation*, 101:1895-1898, 2000). To date, there is
10 no consensus on the use of beta- or gamma-sources and on the choice of medium-energy or higher beta energy (Coursey and Ravinder, *Physics Today*, vol. 53(4):25-30, 2000) to prevent restenosis. However, beta-emitter source (i.e., ^{32}P , ^{90}Y , $^{90}\text{Sr/Y}$) significantly reduces
15 operator exposure compared with previous trials with the gamma-emitter isotopes (^{192}Ir). Compared to brachytherapy approach, stent-based radiotherapy acts by preventing both vessel shrinkage and excessive neointimal proliferation.

20 One of the main limitations of the extensive use of radioactive stent in interventional cardiology is the complex clinical prescription of the metallic prosthesis (diameter, length, type, etc.) associated with the choice of the radioisotope and the activity
25 in function of the physical half-life. Regarding those specifications, the production of an active inventory of such device in a daily practice can be difficult and problematic. A major difficulty to overcome is the need to load any pre-manufactured
30 stents with defined amounts of radioactivity at the time of use. Using stents that are preloaded by the

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manufacturer is not ideal because the stent specifications (specific radioactivity, length diameter, etc.) may differ from the need.

Häfelí et al. (*Biomaterials* 19:925-933, 1998) suggested a method for electrodepositing Rhenium (^{186}Re or ^{188}Re) on a stent. However, Häfelí et al. teach that rhenium alone do not electroporate well by itself, and that they had to co-deposit the rhenium with cobalt. Again co-deposition with cobalt caused cracking and flaking of the deposited layer. To overcome these problems, Häfelí et al. deposited over the layer of cobalt rhenium previously deposited a second layer of gold to overlay cobalt and thus prevent cracking. Häfelí et al. also teach that gold, being a noble metal compete with rhenium during the deposition such that gold is deposited preferentially over rhenium.

In International Publication WO 98/17331, there is disclosed an implantable medical device on which a bioactive material may be deposited thereon and retained with a porous layer deposited over the bioactive material layer. However, such procedure is complicated and may not in every cases be reliable and reproducible.

International Publication WO 98/23299 only discloses the preparation and use of a radiolabeled DNA oligonucleotide, without further providing any method for preparing an angioplastic device as described in the present application.

Furthermore, International Publication WO 99/02195 describes a stent with a radioactive,

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radiopaque coating. However, the radioactivity needs to be deposited on the radiopaque material, which itself is deposited on the stent, rendering the method more complicated than the one disclosed hereinafter in the present application.

Consequently, it would be highly desirable to be provided with a strong and rapid deposition process of radioactivity emitting source (such as ^{32}P -oligonucleotide based) on the surface of a device such as a stent to prevent restenosis post-angioplasty, and that would not crack or flake. The ability of ^{32}P -labeled oligonucleotide to inhibit neointimal hyperplasia was already demonstrated in an *in vitro* model (Fareh et al., *Circulation*, 99:1477-1484, 1999).

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SUMMARY OF THE INVENTION

One aim of the present invention is to provide a strong and rapid deposition process of radioactive

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molecule on the surface of an angioplastic device for preventing restenosis post-angioplasty.

In accordance with the present invention there is provided a method for depositing a charged molecule on an angioplastic device. This method comprises the step of contacting the angioplastic device with a solution containing the charged molecule under suitable conditions for deposition of the charged molecule on the angioplastic device. The charged molecule is preferably a radioactive charged molecule.

The deposition can be passive or active. By active deposition, it is meant to comprise electrodeposition.

In passive deposition, the angioplastic device has preferably stainless steel or gold on its surface. For gold surface, the charged molecule preferably comprises a thiol-containing group for attaching to the gold on the angioplastic device. For stainless steel, the surface is preferably coated with silicon oxide (SiO_2) or silicon (Si) to be modified with chemical or electrochemical treatments for its functionalization. Stainless steel surface can be also directly used for electrochemical functionalization.

Also in accordance with the present invention, there is provided a method for immobilizing a charged molecule on an angioplastic device using passive deposition or electrodeposition. For the electric approach (electrodeposition), the method comprises the step of applying an electric potential difference between the angioplastic device and a solution

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containing the charged molecule, said charged molecule having a charge opposite to the electric potential difference and being thereby electrodeposited on the angioplastic device.

- 5 The electric potential difference can be made positive or negative, depending on the charge of the molecule to be coated on the device.

 Preferably the radioactive molecule comprises a β -emitter. Preferred β -emitters are selected from the group consisting of Antimony-124, Cesium-134, Cesium-137, Calcium-45, Calcium-47, Cerium 141, Chlorine-36, Cobalt-60, Europium-152, Gold-198, Hafnium-181, Holmium-166, Iodine-131, Iridium-192, Iron-59, Lutetium-177, Mercury-203, Neodymium-147, Nickel-63, Phosphorus-32, Phosphorus-33, Rhenium-186, Rhodium-106, Rubidium-86, Ruthenium-106, Samarium-153, Scandium-46, Silver-110m, Strontium-89, Strontium-90, Sulfur-35, Technetium-99, Terbium-160, Thulium-170, Tungsten-188, Yttrium-90 and Xenon-133.

- 20 When the electric potential difference applied is positive, the radioactive molecule is preferably selected from the group consisting of a radioactive DNA or an analog thereof, a radioactive RNA, a radioactive nucleotide, a radioactive oligonucleotide, radiactive H_3PO_4 , radioactive diethylenetriaminepenta-25 acetic acid, and a radiactive polyanionic complex. More preferably the radioactive molecule is a radioactive oligonucleotide. The oligonucleotide is preferably a 8- to 35-mer oligonucleotide, more preferably a 8- to 20-mer oligonucleotide, and most 30 preferably a 15-mer oligonucleotide. These molecules

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form negative ions in solutions and are therefore attracted onto the angioplastic device.

When the electric potential difference applied is negative, molecules are preferably selected from the group consisting of conjugated cationic polypeptides, cationic peptides, dextran, polyamines and chitosan. These molecules are preferably radioactive molecules. These molecules form positive ions in solutions and are therefore attracted onto the angioplastic device.

The angioplastic device may be for example a stent. Preferably the angioplastic device has a metallic surface, such as stainless steel, gold, tantalum, nickel and titanium or any alloy thereof.

The method of the present invention may further comprise before the step of applying an electric potential difference, a step of surface cleaning of the angioplastic device with a solvent, electrochemical or argon-ion sputtering treatments for removing impurities at the surface of said angioplastic device, or, after the step of applying an electric potential difference, a further step of rinsing the angioplastic device for removing free molecule at the surface of said angioplastic device.

In a preferred embodiment of the present invention, the surface of the angioplastic device is fonctionnalized for molecule coating. The angioplastic device may be fonctionnalized for example with a diazonium treatment.

Still in accordance with the present invention, there is provided an angioplastic device for

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preventing restenosis in a coronary and/or peripheral artery, said device comprising a radioactive charged molecule deposited on its surface.

Further in accordance with the present invention, there is provided a method for preventing restenosis in a coronary and/or peripheral artery comprising implanting an angioplastic device as defined above at a site of potential restenosis such as coronary and/or peripheral artery in a patient in need of such a treatment.

The method of the present invention is rapid and allows obtaining a radioactively coated device, on which a radioisotope-containing molecule is effectively and uniformly deposited. No adverse effects of deposition treatment are observed in coated stent *in vitro* (mechanical and colorless properties) and *in vivo* (clotting, thrombogenicity). Strong and effective binding of ^{32}P -oligonucleotides on metallic surface was obtained.

Since the method of the present invention is rapid, it also allows to use simultaneously a stent with radiotherapy for preventing restenosis. It is now possible with the method of the present invention to attach a radioisotope-carrying molecule on a device such as a stent, according to a simple method. The simplicity of the method allows for that method to prepare a radioactively coated stent to be used for implantation just moments after its preparation.

By the term functionalization, it is intended to mean the application of a reagent to a solid surface that will permit molecule coating. By the

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term radioactively coated device, it is intended to mean any device used in the art for treating restenosis. Such device can be without limitation a stent or a radioactive filament for radiotherapy at the site of restenosis or at the site of angioplasty for preventing restenosis in coronary or peripheral vessels.

By the term angioplastic device, it is intended to mean any device used for angioplasty for which radiotherapy would be beneficial. Such device may be without limitation a stent or a wire or any other device to which a person of the art may think of for the prevention of an uncontrolled proliferative lesion. The term angioplastic device is also meant to include any prosthesis to be implanted within a vessel or within other body conduit such as, but not restricted to, the bile duct or urethra for the purpose of endovascular treatment.

By the term analog of DNA, it is intended to mean nucleic acid sequences such as double-strand DNA sequences, single-strand DNA sequences, RNA or any combination thereof.

By the term radioactive polyanionic complex, it is intended to mean a molecule carrying at least one radioactive element and bearing at least one negative charge.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the nature of the invention, reference will now be made to the

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accompanying drawings, showing by way of illustration a preferred embodiment thereof, and wherein:

Fig. 1 illustrates a schematic electrodeposition set-up in accordance with a preferred embodiment of the present invention;

Fig. 2 is a schematic reaction chamber for glicidoxy-propyltriethoxy silane (GPTS) modification for passive deposition;

Fig. 3 illustrates a schematic electrodeposition set-up used for diazonium functionalization of silicon and stainless steel surfaces for passive deposition;

Fig. 4 shows the effect of duration of passive deposition of ^{32}P -oligonucleotide on bromobenzenediazonium coated stainless steel surface;

Fig. 5 is a line graph of electrodeposition of 15-mer oligonucleotide on gold electrode as a function of potential;

Fig. 6 illustrates the adsorption isotherm of 15-mer oligonucleotide on gold electrode at different pH of the electrolyte solutions;

Fig. 7 illustrates the adsorption isotherm of 8-mer oligonucleotide at different concentrations on gold electrode;

Fig. 8 illustrates the effect of duration of polarization on the level of coating of radioactive 15-mer oligonucleotide onto gold plated stent;

Fig. 9 illustrates the effect of increasing activity of radioactive 15-mer oligonucleotide on coating onto gold plated stent;

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Fig. 10 illustrates the effect of duration of polarization on the level of coating of radioactive 15-mer oligonucleotide onto stainless steel stent;

Fig. 11 illustrates the effect of increasing activity of radioactive 15-mer oligonucleotide on coating onto stainless steel stent;

Fig. 12 is a scan graph of gold plated stents coated with the electrochemical method of the present invention illustrating the distribution of the radioactive molecules onto the metallic surface along the length of the stent;

Fig. 13 is a scan graph of stainless steel stents coated with the electrochemical method of the present invention illustrating the distribution of the radioactive molecules onto the metallic surface along the length of the stent;

Fig. 14 is a line graph of the in vitro retention profile of ^{32}P -oligonucleotide coated onto the surface of a gold plated stent;

Fig. 15 is a line graph of the in vitro retention profile of ^{32}P -oligonucleotide coated onto the surface of a stainless steel stent;

Fig. 16 is a line graph of the retention profile of ^{32}P -oligonucleotide-coated gold stent (16 mm) when implanted in porcine coronary; and

Fig. 17 is a line graph of the retention profile of ^{32}P -oligonucleotide-stainless steel stent (18 mm) when implanted in porcine coronary artery.

30 DETAILED DESCRIPTION OF THE INVENTION

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In accordance with the present invention, there is provided a method for electrodepositing a radioactive molecule on a device for preventing restenosis.

5 In a preferred embodiment of the invention, the deposition is an electrodeposition as illustrated in Fig. 1 with the potentiostat/Galvanostat (EG&G model 273A) 20, hereinafter referred to as the potentiostat. In fact, Fig. 1 illustrates the Schematic drawing of
10 the electrochemical cell and angioplastic device used for radioactive molecule coating onto gold and stainless steel surfaces.

In this embodiment, electrodeposition is effected under a nitrogen atmosphere (N_2), in a glass
15 cell 22. The stent 24, which acts as the working electrode, is submerged in the electrolyte 26 with a reference electrode 28 (preferably a PdH_2 electrode) and a counter electrode 30 (Pt plate). The three electrodes are connected to the potentiostat 20, which
20 is itself connected to a computer 32 for recording the working conditions. The cell 22 is provided with a cover 34 provided with holes for allowing the wires of the electrodes to pass through. The cover 34 is also provided with a gas inlet 36 and a gas outlet 38 for
25 allowing nitrogen to be circulated.

In another preferred embodiment of the invention, the deposition is a passive deposition in which case the set up is similar to the one illustrated in Fig. 1, with the exception that no
30 potentiostat 20 is needed. In such an embodiment, the alternate method of depositing a radioactive

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polyanionic complex, such as a radioactive oligonucleotide, comprises the step of modifying the oligonucleotide by adding a thiol-containing group. The thiol-containing group may be for example a C₆ chain carrying a thiol function at its extremity and which is added at the 5' end of the oligonucleotide. The so-modified oligonucleotide may be labeled with ³²P or other radioactive elements. A gold or gold-coated stent is incubated in either 0.1M potassium phosphate buffer (KH₂PO₄ pH 7.0) or pure tetrahydrofuran containing the radiolabeled oligonucleotide. After a 60 minute incubation period at room temperature, the stent is rinsed with distilled water. The radioactive oligonucleotide attaches to gold by the thiol group, producing a radioactively coated stent. This preferred embodiment is only an example (refer to example I) of passive deposition caused by the high affinity of gold for thiol group.

Another example of passive deposition is based on the surface coating with silicon (Si) or silicon oxyde (SiO₂) followed by surface fonctionnalization with substrates. In this other preferred embodiment of the invention, the SiO₂-treated surface is then modified with glicidoxy-propyltriethoxy silane (GPTS), whereas the Si-treated surface is fonctionnalized with 4-bromobenzenediazonium tetrafluoroborate (diazonium). Stainless steel surface can be directly activated with 4-bromobenzenediazonium tetrafluoroborate without Si/SiO₂ pre-treatment. The GPTS modification is passive (Fig. 2), whereas the diazonium deposition is an electrochemical functionalization, in which case

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the set up is similar to the one illustrated in Fig. 3.

Fig. 2 illustrates a Schematic drawing of the reaction chamber for glicidoxy-propyltriethoxy silane (GPTS) modification of silicon oxyde treated surfaces.

In Fig. 2, the substrates are taken out of the oven they are placed in the various slots of the 2 glass holders 50. Each holder is hooked to the reaction chamber 52 where the silanization will take place. The whole lot is then placed inside a glove box which is under dry N_2 atmosphere. Once inside the glove box the GPTS reaction compounds were then added, in sequence, to the reaction chamber. A magnetic stirring bar 54 is added to the reaction mixture, the reaction chamber is then closed and removed from the glove box. The reaction chamber is connected to a water circulator 56 with temperature control. Stirring is initiated and the reaction is allowed to proceed for 4 hours at $70^\circ C$, under continuous N_2 flow 58 originating from a gas tank.

Fig. 3 illustrates Schematic drawing of the electrochemical cell used for bromobenzenediazonium functionalization of silicon and stainless steel surfaces.

In Fig. 3, the electrochemical cell 22 was a standard three-electrode setup. The reference electrode 28 used was a saturated Calomel electrode (SCE) and the counter electrode 30 was platinum foil (1 cm^2). The bromo-aryldiazonium solution was used as the electrolyte for cyclic voltammetry in order to attach the bromo-aryldiazonium to the surface (0.5 cm^2

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area) of the Si or 316L substrates acting as working electrode 24. A scanning potentiostat was used to apply dc potentials to the working electrodes. The current-voltage response was recorded on an XY
5 recorder.

In this preferred embodiment of the invention, the alternate method of depositing a radioactive polyanionic complex, such as a radioactive oligonucleotide, comprises the step of modifying the
10 oligonucleotide by adding an amine-containing group. The amine-containing group may be for example a C₆ chain carrying an amine function at its extremity and which is added at the 5' end of the oligonucleotide. The so-modified oligonucleotide may be labeled with ³²P
15 or other radioactive elements.

This preferred embodiment is only an example of passive deposition caused by the high affinity of GPTS and diazonium substrates for amine group.

In another embodiment of the invention, the
20 radioisotope can be attached to other radioisotope-carrying molecule.

For instance, in the preferred embodiment of an electrodeposition set-up (Fig. 1) where the stent plays the role of the anode (positively charged), a
25 negatively charged molecule can be used for an effective electrodeposition onto the stent surface. Preferred negatively charged molecules can be for example without limitation labeled DNA or RNA, or labeled analogs thereof, labeled nucleotides,
30 radioactive H₃PO₄, labeled diethylene triamine

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pentaacetic acid (DTPA) or labeled polyanionic complexes.

In another preferred embodiment of an electrodeposition set-up where the stent plays the role of the cathode (negatively charged), a positively charged molecule can be used for an effective electrodeposition onto the stent surface. Such positively charged molecule can be for example without limitation labeled conjugated polypeptides, labeled cationic peptides, labeled dextran, labeled chitosan or labeled polyamines.

In accordance with one embodiment of the invention, there is provided a process that can be performed in a daily practice moments prior to the implantation of the device in a catheterization laboratory or in the radiation oncology department, and administered to the patient according to the specification desired. The vehicle carrying the radioisotopic source such as a beta-source (^{32}P) is preferably a short DNA sequence (15 mer oligonucleotides linked together by 11 phosphorothioate bounds), rendering the molecule stable over a long time. Strong binding of DNA-oligonucleotides was reported on gold (Selligren et al., *Anal. Chem.*, 68(2):402-407, 1996).

When double-stranded nucleic acid is used to be coated on the stent, a first non-radiolabeled strand of this double-stranded nucleic acid can be coated on the stent in accordance with one embodiment of the invention. The second complementary strand of the double-stranded nucleic acid can be labeled and

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annealed to the first strand. Such embodiment is also envisioned by the present invention, and is also encompassed in the term a radioactively coated device.

While a β -emitter source of radioisotope is preferred, other sources of radioisotope can also be used in accordance with the present invention.

The radioisotopic source is determined according to the treatment determined. Depending on the cases, the radiotherapy might vary from one patient to another. Accordingly, the radioisotopic source will be determined based on the half-life of the radioisotopic source, its energy and the specific activity of the radioisotopic source desired. The determination of the radioisotopic source is within the skill of a person of the art.

Preferably the radioactive molecule comprises a β -emitter. Preferred β -emitters are selected from the group consisting of Antimony-124, Cesium-134, Cesium-137, Calcium-45, Calcium-47, Cerium 141, Chlorine-36, Cobalt-60, Europium-152, Gold-198, Hafnium-181, Holmium-166, Iodine-131, Iridium-192, Iron-59, Lutetium-177, Mercury-203, Neodymium-147, Nickel-63, Phosphorus-32, Phosphorus-33, Rhenium-186, Rhodium-106, Rubidium-86, Ruthenium-106, Samarium-153, Scandium-46, Silver-110m, Strontium-89, Strontium-90, Sulfur-35, Technetium-99, Terbium-160, Thulium-170, Tungsten-188, Yttrium-90 and Xenon-133.

Electrodeposition

Stainless steel stent characteristics and surface pre-treatment

In a preferred embodiment, ACS multi-link RX
5 DUET™ stents (Guidant Vascular Intervention, Santa Clara, CA) of 13 to 23 mm of length were used in accordance with the present invention. Commercial 316L stainless steel samples, in the form of 1 cm diameter discs, 0.2 mm thick (Goodfellow Cambridge Ltd.,
10 Huntingdon, England) were also used.

Deposition or electrodeposition is more effective when the surface to be coated is cleaned to remove contaminants. To do so, stents to be coated were first washed with organic solvents (acetone or
15 methanol) for removing contaminants and then air-dried. Another example of surface cleaning is argon-ion sputtering. The sputtering of stents or discs was carried out under the following conditions :

| | | |
|----|-----------------------------------|---------------------------|
| | Initial chamber pressure | 1,3x10 ⁻⁸ torr |
| 20 | Pressure after argon introduction | 1,3x10 ⁻⁵ torr |
| | Energy | 2 keV |
| | Focus voltage | 1 keV |
| | Current | 4 μA |
| | Time | 20 min (discs) |
| 25 | | 5 min (stents) |

Again, transfer of discs and stents was carried out under vacuum.

An electrochemical method can also be used for cleaning stainless steel surface (stents or discs).
30 Electropolishing was carried out in the glove box using a voltage generator. The cleaning solution was composed of 1 M oxalic acid 15% hydrogen peroxide.

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Only two electrodes were used : the sample was one and the other, a Pt disk. A potential of 10 V was applied for 10 minutes between these electrodes, followed by extensive rinsing and transferred to the electrochemical deposition cell of Fig. 1.

Gold stent characteristics and surface pre-treatment

In a preferred embodiment, NIROYAL™ 24 ct gold plated stents (Boston Scientific Ireland Ltd. Ballybrit Business Park, Galway, Ireland) of 13 to 23 mm of length were used in accordance with the present invention. Gold-coated 316L discs in the form of 1 cm diameter discs (Goodfellow) were also used.

Gold surface can be directly used for electrodeposition or cleaned with argon-ion sputtering in conditions as previously described for stainless steel metal.

³²P-oligonucleotide compounds

In one embodiment of the invention, the vehicle chosen for carrying the beta-source (³²P) is a short DNA sequence (15 mer oligonucleotides linked together by 11 phosphorothioate bounds, patent No. 5,821,354). This short DNA sequence was reported to be highly stable and effective in the prevention of cell proliferation with no side effects (Fareh et al., *Circulation*, 99:1477-1484, 1999).

For the embodiment of passive deposition, the radioactive molecule has at its 5' end either an amine-containing group as for example a C₆ chain carrying an amine function or a C₆ chain carrying a thiol function. The amine- and thiol-modified

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oligonucleotides may be labeled with ^{32}P or other radioactive elements.

Electrodeposition of ^{32}P -oligonucleotides

Electrodeposition is effected in an electrochemical cell containing the ^{32}P -oligonucleotides (75 $\mu\text{Ci}/50 \mu\text{L}$ of water) diluted in 250 μL of acetate sodium buffer, ($\text{CH}_3\text{CH}_2\text{CO}_2\text{Na} \cdot 3\text{H}_2\text{O}$ at 0.2 M) at pH 8.5. In the electrochemical cell containing both electrolyte and ^{32}P -oligonucleotide solutions, a metallic stent was fixed to the anode and the cathode was composed of a platinum wire of 2 mm diameter and 5 cm length or a Pt plate.

Electrodeposition is performed by applying a voltage of 1 Volt (50-60 mA) for 15 minutes using a standard potentiostat 20 at room temperature.

Electrodeposition succeeds in binding 2.5% of initial ^{32}P -oligonucleotides on the stent surface, when any post-treatments were applied.

Another example of electrolytes for effective electrodeposition is aqueous phosphate solutions.

To evaluate the electrodeposition of the 15-mer oligonucleotide onto the gold surface (electrodes and plated stents) in aqueous phosphate solutions as electrolytes, a method for studying the adsorption of DNA was used. Briefly, cyclic voltammetry (CV) coupled to electrochemical quartz crystal nanobalance system was used to study the adsorption of organic molecules on gold surface. Since the frequency variation of the crystal and the cyclic voltammogram are recorded simultaneously, this method allows to measure the quantity of molecules adsorbed on gold in the whole

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potential window and in only one cycle. Fig. 5 illustrates a surface concentration (Γ) of 15-mer oligonucleotide ($3.8 \mu\text{M}$) on gold electrodes as a function of potential in the pH-6.98-7.0 phosphate buffered solution. The scan rate is 100 mV/s. An arrow indicates the beginning of the scan.

As illustrated in Fig. 5, the electrosorption of 15-mers increases as the polarization potential is increased and reaches a maximum $E = 1.1-1.2 \text{ V vs. SCE}$ (calomel reference electrode) (see Fig. 5). At potential higher than 1.1-1.2 V, the surface concentration of the molecule starts to decrease. This phenomenon can be explained by the oxidation of gold occurring at these potentials when using phosphate buffer as electrolyte solution.

After repeating the same procedure for several different concentrations of 15-mer molecule, the adsorption isotherm at constant potential was obtained in those conditions. For that example, gold plated stents (NIROYAL™) and commercial electrodes of gold (0.1684 cm^2 , Aldrich Canada) were used. Gold wires were inserted in a Kel-F rod in order to have only one tip of the wirer in contact with the solution. Kel-F was chosen as the support material because it is inert in acidic and basic aqueous media. The electrode was polished with a $0.5 \mu\text{m}$ alumina suspension. Aqueous phosphate solutions were prepared from a $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (17.8897 g/L) and a KH_2PO_4 (9.0725 g/L) solutions.

Fig. 6 illustrates the adsorption isotherm of 15-mer oligonucleotide on gold electrodes at $E=1.1 \text{ V, SCE}$ (calomel reference electrode) in phosphate

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buffered solutions at pH=6.98-7.0, pH=8.04 and pH=5.59. In Fig. 6, the adsorption isotherm of non-radioactive 15-mer oligonucleotide on gold at 1.1 V vs. SCE is presented for the three buffered solutions studied. One may note that at pH=6.98-7.0, an increase of the concentration of oligonucleotide leads to an increase of the surface concentration, until a plateau is attained at a concentration of about 20 μM . Beyond this point, an increase in the concentration of 15-mer oligonucleotide does not enhance the surface concentration. Similar experiments were performed at pH=5.59 and pH=8.04 showing that electrosorption of 15-mer oligonucleotide on gold is more effective at pH=6.98-7.0. Higher electrosorption was obtained when polarization was performed at 60°C.

Fig. 7 illustrates the adsorption isotherm of 8-mer oligonucleotide on gold electrodes at $E=1.1$ V, SCE (calomel reference electrode) in phosphate buffered solutions at pH=6.98-7.0. As shown in Fig. 7, electrosorption of 8-mer oligonucleotides is effective onto gold electrode surface, when applying a voltage of 1.2 V during 15 minutes at room temperature. Higher electrosorption was obtained when polarization was performed at 60°C. Similar adsorption isotherm of a 35-mer oligonucleotide was reported.

When gold stents (16 mm) were polarized at 1.2 V during 15 to 30 minutes in presence of ^{32}P -oligonucleotide (800 μCi) at room temperature, 2.5 to 3 μCi of radioactivity were detected onto the stent surface (corresponding to 0.3% of efficacy coating)

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and no alteration of the surface integrity was reported.

Other electrolyte useful for the present invention

³²P-oligonucleotide depositions were carried out in 0,1 M HClO₄ under nitrogen (bubbler), at a potential of 1,45 V vs. SCE (saturated calomel electrode) and at a temperature of 60 ± 10 °C. For that preferred embodiment, higher coating was obtained at 60°C. However, coating of ³²P-oligonucleotide onto stainless steel or gold surfaces is also feasible and effective at room temperature.

The electrochemical cell (Fig. 1) was composed of three electrodes : i) the working electrode (our sample); ii) the counter electrode (Pt disk); and the reference electrode (Pd/PdH₂), calibrated before each measurement.

The reference electrode is made by flowing hydrogen on a Pd disk in 0,1 M HClO₄ for 30 minutes.

The effects of polarization duration and the initial activity were assessed with native gold stents of 16 mm, where no surface cleaning was performed. Similarly, stainless steel stent of 18 mm, previously cleaned with 1M oxalic acid 15% hydrogen peroxide were also used. A series of time of electrodeposition (5, 15, 30 and 60 min) were used.

Fig. 8 illustrates the effect of coating duration on electrodeposition level (16 mm-gold plated stents). As illustrated on Fig. 8, the maximal coating was reached at 5 to 15 min on gold surface, underlying the rapid and effective electrodeposition of ³²P-oligonucleotide onto the gold stent (average of

1.6%). Fig. 9 reports the activity-dependent coating onto gold surface when increasing activity of ^{32}P -oligonucleotide (0.25, 0.5, 1 and 2 mCi) were tested during 5 minutes. However, higher effective coating was obtained at low initial activity (1.9%, 1.2%, 0.8% and 0.5% for 0.250, 0.500, 1.0 and 2.0 mCi respectively). In those conditions (5 min of coating), an effective coating of 0.5% (average) was obtained, corresponding, for example, of an activity of 10 μCi onto a 16 mm-gold stent. Similar levels were obtained when the gold surface was cleaned with argon-ion sputtering.

Fig. 10 illustrates the effect of coating duration on electrodeposition level (18 mm-stainless steel stents). As illustrated in Fig. 10, a similar coating of 0.5% was obtained at 5 to 15-20 min to reach a maximal coating (1.0%) at 60 min on stainless steel surface, underlying the rapid and effective electrodeposition of ^{32}P -oligonucleotide onto the stainless steel stent. When increasing activity of ^{32}P -oligonucleotide (0.25, 0.5, 1 and 2 mCi) were tested, similar coating with activity of 0.25 to 1.0 mCi (average of 2.5-3.0 μCi) were obtained, whereas higher activity (2.0 mCi) led to significant amount of ^{32}P -oligonucleotide onto the stainless steel stent surface (Fig. 11). In those conditions (15 min of coating), an effective coating of 0.5% (average) was obtained, corresponding, for example, of an activity of 10 μCi onto a 18 mm-stainless steel stent. Fig. 11 illustrates the effect of increasing activity of ^{32}P -oligonucleotide on coating efficiency (18 mm-stainless

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steel stents). Similar levels were obtained when the stainless steel surface was cleaned with argon-ion sputtering.

³²P-oligonucleotide distribution onto the surface

5 Coated stents (n=6 gold plated and n=6 stainless steel stents), using HClO₄ as electrolytes, were scanned for 4 hours to visualize the distribution of ³²P-oligonucleotides onto the metallic surface along the length of the stent. The stent radiation uniformity
10 was measured using a 0.5 mm slit in front of a Geiger counter which was moved over the stent in 0.5 mm steps by a computer-controlled stepping motor.

Regarding the scan graph of the coated stent, the electrodeposition was highly uniform on the
15 metallic surface of gold plated (Fig. 9) and stainless steel stents (Figs. 12 and 13). Figs 12 and 13 illustrate scan graphs of a gold plated stent or of a stainless steel stent, respectively, coated with ³²P-oligonucleotide.

20 Similar uniform distribution of radioactivity was also obtained when acetate sodium buffer as electrolytes was used to perform electrodeposition in the set-up of Fig. 1.

25 Post-treatment of the radioactive stents (in vitro retention)

Following electrodeposition in the acetate sodium buffer electrolyte, radioactive stents were rinsed in distilled water for 24 hours at room temperature and air-dried or sonicated for 30 minutes.
30 Biological treatments were investigated by incubating radioactive stents with DMEM supplemented with an enzyme solution consisting of 5 µl of Nuclease S₁

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(332 U/ μ l), 1 μ l of Exonuclease III (E. coli; 100 U/ μ l), and 1 μ l of phosphodiesterase (0.5 U/ μ l) in presence of 10% Fetal Bovine Serum (FBS, Gibco) overnight at 37°C. Following incubation of coated
5 stents in water for 24 hours, 80% of initial coating solution remained on the metallic surface, whereas additional sonication procedure (30 minutes) reduced to 50% the retention rate. Following a biological treatment (blood mimicking enzyme solution) of coated
10 stents at 37°C during 14 to 16 hours, 12% of the amount of radioactivity remained on the stent, when compared to the initial electrodeposition level.

Following electrodeposition in the HClO₄ 0.1M electrolyte, radioactive coated stents (n=8 gold
15 plated stents of 16 mm) were incubated in biological medium composed of DMEM in presence of 20% Fetal Bovine Serum (FBS, Gibco) at 37°C with constant agitation. Those physical and biological conditions were used to mimic *in vivo* conditions. A sample of
20 medium (50 μ L) was counted following 15, 30, 60, 120, 240 min and 24 hours of incubation. Fig. 14 illustrates the retention profile of coated ³²P-oligonucleotide onto 16 mm-gold plated stent surface in *in vitro* conditions (blood mimicking conditions).
25 As illustrated in Fig. 14, following incubation of gold coated stents at 37°C, a progressive elution of the ³²P-oligonucleotide was reported, corresponding to a remaining activity of an average of 50, 40 and 35% after 60, 120 and 240 min respectively. A significant
30 sustained activity of 10-12% is reported up to 8 days of treatment in blood mimicking conditions, when

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compared to the initial electrodeposition level (Fig. 14).

Similarly, radioactive coated stents (n=8 stainless steel stents of 18 mm) were incubated in
5 biological medium composed of DMEM in presence of 20% Fetal Bovine Serum (FBS, Gibco) at 37°C with constant agitation. A sample of medium (50 µL) was counted following 15, 30, 60, 120, 240 min and 18 hours of incubation. Fig. 15 illustrates the retention profile
10 of coated ^{32}P -oligonucleotide onto 18 mm-stainless steel stent surface in in vitro conditions (blood mimicking conditions). As illustrated in Fig. 15, following incubation of stainless steel coated stents at 37°C, a progressive elution of the ^{32}P -
15 oligonucleotide was reported, corresponding to a remaining activity of an average of 45 to 37-40% after 60 to 240 min. A significant sustained activity of 40% is reported following 1 day of treatment in blood mimicking conditions; an average of less than 10% of
20 initial electrodeposition level remained up to 7 days of incubation.

Regarding the combination of a simple method to produce radioactive stent and a well defined release of the radioactive molecule from the angioplastic
25 device, a classical stent-based radiation as well as a stent-based pharmacological approach can be envisaged to prevent restenosis.

To reinforce the strength of the proposed radioactive coating, the metallic surface can be
30 embedded in a simple manner. A series of biostable coatings and agar solution of 1 to 2% were tested and

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shown to improve the molecule retention by reducing the elimination of the ^{32}P -oligonucleotide from the metallic surface. Polymer coating (such as parylene) already used for medical application is proposed to
5 embed the angioplastic device.

To support the pharmacological approach, the well-defined elution from the coated stents can serve as a local drug delivery device to prevent restenosis, based on data obtained on intra-arterial sustained-
10 release of beta particles. In that case no device embedding is performed.

Mechanical properties of the radioactive coated stents

General observations were done on the coated stents such as determination of color and rigidity.
15 Mechanical properties were estimated by mimicking in vivo stent deployment. After mounting the stent on deflated balloon, the balloon was inflated to 10-14 atm and the capability of stent deployment was evaluated. No physical alteration (color and
20 deployment ability, surface deterioration, cracking and flaking of the surface) was observed in coated stents according to the present invention. Under fluoroscopy, the visibility of the coated stent was not modified.

25 Implantation of the radioactive coated stent in porcine coronary arteries

Domestic pigs were sedated with intramuscular injection of ketamin, azaperon and atropine to undergo anesthesia with thiopental sodium (iv). The pigs were
30 intubated and ventilated with a mix of isoflurane 2% and oxygen during the procedure. An 8 Fr. guiding catheter was advanced through a femoral sheath with a

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0.035 J guide-wire, under fluoroscopic monitoring in the ascending aorta. The guide wire was then removed, allowing the guiding catheter to be positioned in the ostium of the target vessel. Prior to performing the angiography, a bolus of 1 ml of nitroglycerin solution with a concentration of 0.3 mg/mL is injected intra-coronary. The angiography was then performed in at least two near orthogonal views that visualize the target site of right coronary artery (RCA) or left circumflex artery (LCX) of the pig. A quantitative coronary angiography (QCA) measure was done to assess the vessel size for adequate stent implantation. Stent was advanced to the target site and balloon inflation at 10 to 12 atm for 30 seconds was performed to adequately deploy the stent (2 stents per pig). Following stent implantation, the balloon was deflated and the catheter withdrawn. Control angiography was then performed to document any residual luminal stenosis or vessel wall dissection. If spasm was documented, 1 ml of nitroglycerin solution at a concentration of 0.3 mg/mL was injected intra-coronary.

Macroscopical observations

After stent implantation, treated pigs were maintained for 6 hours under observation. Following pig euthanasia with a lethal dose of KCl, myocardium was dissected to remove stented arteries. A macroscopical observation of the heart and stented artery was performed to explore the potential side effects of coating stent implantation (thrombogenicity, clotting, etc.). Stents were then

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removed from the artery to be counted to assess the in vivo retention of ^{32}P -oligonucleotides onto the stent surface. For that example, coated-stents generated with acetate sodium buffer as electrolytes and Fig. 1 as electrochemical set-up were used for coronary implantation

Following fluoroscopy and macroscopical observations, no side effects related to the implantation of a radioactive treated stent according to the present invention were observed either in the myocardial tissue or in the implanted artery. Measurements of radioactivity level of coated stents revealed that 6 hours following stent implantation 45% of initial coated activity remained on the stent surface, whereas low radioactivity was detected in the target artery (less than 3%), suggesting that coronary wash-out eliminates more than 44% of the drug from the stent surface within 6 hours. The biological half-life of coated ^{32}P -oligonucleotides on the surface stent in porcine coronary arteries was estimated to be approximately 5.5 to 6 hours. The residence time of the coated ^{32}P -oligonucleotides is 11- to 12-fold higher than direct intra-mural administration of liquid ^{32}P -oligonucleotides using the Infiltrator® catheter (0.51 hours).

In vivo follow-up of ^{32}P -oligonucleotide elution from coated stents

The catheter-based radiation detection via the endovascular detector permits the fine and continuous determination of the elution profile of the radioactive molecule from the stent. For that issue, gold-plated (16 mm) and stainless steel (18 mm) stents

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were used. ^{32}P -coated stents, generated with HClO_4 as electrolytes, were implanted in porcine coronary arteries (LCX and RCA) for 3 hours as previously described.

5 Using the endovascular detector, measurements of radioactivity levels were done every 30 seconds to follow local ^{32}P -oligonucleotide elution from the stent. At the end of continuous endovascular monitoring (up to 3 hours), the pig was sacrificed
10 with a lethal dose of KCl, myocardium was dissected to remove stented arteries. Blood was collected during the experiment.

 Figs. 16 and 17 illustrate the retention profile of coated ^{32}P -oligonucleotide gold-plated stent
15 (16 mm) and coated ^{32}P -oligonucleotide stainless steel stent (18 mm), respectively, when implanted in porcine coronary artery. As illustrated in Figs. 16 and 17, the elution profile of gold-plated and stainless steel stents, electrocoated with ^{32}P -oligonucleotide, is
20 characterized by two components: a rapid elution during the first 30 min. and a significant sustained radioactivity, which is maintained up to 3 hours. Few radioactivity was detected in blood samples, stented coronary and the adjacent myocardium.

25 The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

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EXAMPLE IPassive Deposition Using Thiol-modified
Oligonucleotide

5 Coating of gold-plated stents with ^{32}P -oligonucleotide
containing a 5'-end thiol moiety.

NIROYALTM gold-stents (6 mm) were placed in a
piranha solution (3:7 v/v, 30% H_2O_2 : 98% H_2SO_4) at 70°C
for 20 min. Stents were then washed with H_2O , acetone,
ethanol and H_2O and dried under a stream of N_2 gas. The
10 pre-cleaned stents were then placed either in
potassium phosphate buffer (K_2HPO_4 - KH_2PO_4 ; pH 7.0) or
in tetrahydrofuran (THF) containing 100 μCi of ^{32}P -
oligonucleotide containing a 5'-end thiol moiety to be
incubated 60 min. at room temperature. Radioactive
15 stents were then rinsed 3 times with 50 ml of H_2O .

Radioactivity levels of NIROYALTM gold stents
following passive deposition was 1.15 μCi when
incubated in pure tetrahydrofuran and 0.02 μCi when
incubated in potassium phosphate buffer, corresponding
20 to an efficiency of passive deposition of 1.15% and
0.02% respectively. Following immobilization, stents
were incubated 2 days in pig blood at 37°C with
constant agitation. Stents were then removed from
biological conditions to be rinsed with water and
25 remaining radioactivity was assessed by scintillation
counting. NIROYALTM gold stents incubated in the
tetrahydrofuran solution supplemented with ^{32}P -
oligonucleotide lost 33% (0.80 μCi residual activity)
and 66% (0.34 μCi residual activity) of its initial
30 activity after 1 and 2 days of incubation,
respectively. Stents incubated in potassium phosphate

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buffer lost 100% of their initial activity after 1 day of incubation.

EXAMPLE II

5 Passive Deposition Using GPTS Modification

Functionalization of Si/SiO₂ substrates using glicidoxypropyltriethoxy silane (GPTS)

- Substrates:

10 The Si/SiO₂ substrates were 1 cm x 1 cm plates taken from diced 4 inch wafers (Tronics Microsystems, Grenoble). The Si (100) is n-type, phosphorous doped to a density of 10¹⁵ cm⁻³, and has a thickness of 0.3 μm. The Si is covered with a thermally grown SiO₂ layer which is 150 Å thick. The back of the Si plates was
15 covered with a Cr/Au ohmic contact.

- Cleaning:

20 The substrates were placed in boiling acetone (Sprectrograde, Aldrich) for 5 minutes, followed by another 5 minutes in boiling methanol (Sprectrograde, Aldrich). The substrates were then dipped in sulfochromic acid (prepared by adding 95 mL of concentrated sulfuric acid (H₂SO₄) to 5 mL of a saturated aqueous solution of potassium dichromate (K₂Cr₂O₇)) for 4 minutes at room temperature.

25 The substrates were rinsed for 15 seconds with distilled-deionized (d-d) water, and then placed in boiling d-d water for 10 minutes. Following this, the substrates were dried with N₂ flow and placed in a clean oven (ambient atmosphere) at 140°C for 1 hour.

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- GPTS modification:

The substrates, with the reaction chamber illustrated in Fig. 2, are placed inside a glove box which is under dry N₂ atmosphere. Once inside the glove box, the substrates were placed in the reaction chamber and the GPTS reaction compounds were then added, in sequence, to the reaction chamber. The reaction mixture consisted of 111 mL of o-xylene (98% sealed under nitrogen, Aldrich), followed by 12.5 mL of GPTS (98% purity, Fluka), and then 1.5 mL of diisopropyl-ethyl amine (99.5% purity sealed under nitrogen, Aldrich) (for a batch of 8 substrates). A magnetic stirring bar is added to the reaction mixture, the reaction chamber is then closed and removed from the glove box.

The reaction chamber is connected to a water circulator with temperature control. Stirring is initiated and the reaction is allowed to proceed for 4 hours at 70°C, under continuous N₂ flow.

The substrates are removed from the reaction chamber, dipped in ethanol (Spectrograde, Aldrich) for 5 minutes (at room temperature), and allowed to dry under ambient atmosphere. The substrates are finally stored individually in glass vials containing 5 mL of ethyl ether (99.9% purity HPLC grade, Aldrich).

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Immobilization of ^{32}P -oligonucleotide onto GPTS modified Si/SiO₂ substrates

^{32}P -oligonucleotide (40 μCi , with or without a C₆ amino linker at the 5' end) is directly deposited onto the surface of a GPTS modified substrate. The ^{32}P -oligonucleotide solution was left to react for 2 hours on the GPTS surface in 0.01M in KOH, under humid atmosphere. The substrate surface was then rinsed with d-d water.

10 **Results**

When passive deposition was performed on Si/SiO₂ substrates fonctionnalized with GPTS, a 5-fold increase of coating was obtained with the ^{32}P -oligonucleotide with amino linker, when compared to simple ^{32}P -oligonucleotide (0.10% vs 0.02% of initial activity, respectively), corresponding to a better affinity of ^{32}P -oligonucleotide with amino linker to the GPTS surface than the non-modified ^{32}P -oligonucleotide. Moreover, the radioactivity level due to immobilized ^{32}P -oligonucleotide with amino linker increases with initial ^{32}P -oligonucleotide concentration up to 300 μCi , at which point it appears to level off. Immobilization efficiency was better at a reaction temperature of 52°C (2.19% of initial activity), when compared to 22°C (0.16% of initial activity), 37°C (0.19% of initial activity) and 70°C (1.0% of initial activity). A 12 to 13 fold-increase of coating was reported when deposition was performed at 52°C, when compared to room temperature conditions.

EXAMPLE IIIPassive Deposition Using Diazonium Modification

Electrochemical functionalization of Si and stainless steel substrates (discs and stents) with bromobenzenediazonium, and ^{32}P -oligonucleotide immobilization

The procedure used to electrochemically modify the Si and the 316L Stainless Steel substrates is described in C. Henry de Villeneuve et al., (*J. Phys. Chem. B*, 101, 2415-2419 (1997)).

Purity of chemicals and solvents

| Chemicals | Source | Purity/Concentration |
|--|-----------------------|----------------------|
| Trichloroethylene | Fisher Scientific | Reagent Grade |
| Ammonium Fluoride | J. T. Baker Chem. Co. | 40% Solution |
| Methanol | EM Scientific | HPLC Grade |
| Acetone | Fisher Scientific | HPLC Grade |
| Hydrofluoric Acid | Fisher Scientific | 49% |
| 4-Bromobenzene-diazonium Tetrafluoroborate | Aldrich Chem. Co. | 96% |
| Sulfuric Acid | Mallinckrodt | 96% |

Substrates:

The silicon (Si, 100) substrates were $1 \times 1 \text{ cm}^2$, taken from a diced wafer purchased from Tronics Microsystems (Grenoble, France). The Si was phosphorous doped (n-type) to a density of 10^{15} cm^{-3} . A gold/chromium film was deposited under vacuum at the backside of the Si substrate providing an ohmic contact. The stainless steel substrates were 316L type

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(Fe/Cr18/Ni10/Mo3), 10 mm in diameter and 0.2 mm thick, from Goodfellow Cambridge Ltd. (Huntingdon, England). In a preferred embodiment, ACS multi-link RX DUET™ stents (Guidant Vascular Intervention, Santa Clara, CA) of 18 mm of length were used in accordance with the present invention. Stents were cut to have a 9 mm of length for experiments.

Prior to the electrochemical functionalization, both types of substrates were submitted to a cleaning/etching procedure. The Si substrates were cleaned by immersing in trichloroethylene, acetone, and methanol for 1 minute each, respectively. They were rinsed in distilled-deionized (d-d) water and dried with N₂ flow. The Si substrates were then chemically etched for one minute in hydrofluoric acid and six minutes in buffered ammonium fluoride, rinsed once again and dried using N₂. The 316L substrates (discs and stents) were immersed in 50 mL of aqua regia (concentrated HCl:HNO₃, 4:1 (v/v)) for 1 minute, rinsed with d-d water and dried with N₂ flow.

Bromo-aryldiazonium salt solution

A 20mM aqueous solution of 4-bromobenzenediazonium tetrafluoroborate in 0.1M H₂SO₄ and 2% HF was prepared by dissolving 0.54g of 4-bromobenzenediazonium tetrafluoroborate, 0.56 mL of concentrated H₂SO₄ and 4mL of concentrated HF in 100 mL of d-d water. The solution was deaerated by bubbling N₂ for approximately 20 minutes.

Electrochemical functionalization:

The electrochemical cell was a standard three-electrode setup. The reference electrode used was a

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saturated Calomel electrode (SCE) purchased from Fisher Scientific and the counter electrode was platinum foil (1 cm²). The electrochemical cell is illustrated in Fig. 3.

5 The bromo-aryldiazonium solution was used as the electrolyte for cyclic voltammetry in order to attach the bromo-aryldiazonium to the surface of the Si or 316L substrates acting as working electrode. A scanning potentiostat (EG&G Princeton Applied Research
10 Model 362) was used to apply dc potentials to the working electrodes. The current-voltage response was recorded on an XY recorder (Phillips, Model PM 8143).

A single-cycle voltammogram was run on each substrate. The current range was set at 1mA. The
15 reductive scan was run from an initial potential of - 0.3 V to a final potential of - 1.9 V vs. SCE, and back. The scan rate was set at 100 mV/s. A typical reductive wave (at ~ -1.5 V) was observed for modification of a Si substrate. The current density is
20 greater for the 316L substrate because of its greater conductivity and the reduction wave is observed at ~ - 0.95 vs. SCE.

Results

In that series of experiments, all stainless
25 steel surface (discs and stents) were functionnalized with diazonium and then coated in presence of 50 µL (50 µCi) of ³²P-oligonucleotide/amino linker solution. They were rinsed as previously described. ³²P-oligonucleotide with a C6 amino linker at the 5' end
30 was used for that embodiment.

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Using the discs surface, immobilization efficiency reached a level of $9.5 \mu\text{Ci}/\text{cm}^2$ with initial activity of $50 \mu\text{Ci}$ of ^{32}P -oligonucleotide/amine linker solution (9.5% of efficiency). Increasing initial activity to $300 \mu\text{Ci}$ improved the coating efficiency to $15.8 \mu\text{Ci}/\text{cm}^2$. Coating was better at a reaction temperature of 52°C , when compared to 22 and 70°C . A 2 to 3 fold-increase of coating was reported when deposition was performed at 52°C (8 to $18 \mu\text{Ci}/\text{cm}^2$), when compared to room temperature conditions. As shown in Fig. 4, the level of coating increased with the reaction time (5 , 15 , 30 , 60 and 120 minutes). The radioactivity undergoes a gradual increase with reaction time, going from approximately $6 \mu\text{Ci}/\text{cm}^2$ at 5 minutes to $17.5 \mu\text{Ci}/\text{cm}^2$ at 120 minutes. When compared to disk functionalization, immobilization efficiency was increased by 1.4 fold on stainless steel stent surface. An average of $2.93 \mu\text{Ci}$ of ^{32}P -oligonucleotide/amino linker solution was coated on a stainless steel stent of 9 mm , corresponding to a level of $24.5 \mu\text{Ci}/\text{cm}^2$ or an activity of $5.9 \mu\text{Ci}$ for a stent of 18 mm . Those experimental conditions underlined the rapidity of the coating of ^{32}P -oligonucleotide/amino linker solution of the stent surface.

Fig. 4 illustrates the effect of duration of passive deposition on ^{32}P -oligonucleotide coating onto bromobenzenediazonium-treated stainless steel surface.

While the invention has been described in connection with specific embodiments thereof, it will

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be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

What is claimed is:

1. A method for depositing a charged molecule on an angioplastic device, said method consisting essentially of the step of contacting the angioplastic device with a solution containing the charged molecule under suitable conditions for deposition of the charged molecule on the angioplastic device.
2. The method of claim 1, wherein the deposition is a passive deposition.
3. The method of claim 2, wherein the angioplastic device has gold on its surface, and wherein the charged molecule comprises a thiol-containing group for attaching to the gold on the angioplastic device.
4. The method of claim 1, wherein the deposition is an electrodeposition.
5. A method for electrodepositing a charged molecule on an angioplastic device, said method consisting essentially of the step of applying a charge to said angioplastic device for depositing the charged molecule, said charged molecule having a charge opposite to the charge of the angioplastic device to thereby electrodeposit the charged molecule on the angioplastic device.

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6. The method of claim 5, wherein the charge of the angioplastic device is positive.

7. The method of claim 5, wherein the charge of the angioplastic device is negative.

8. The method of claim 7, wherein the radioactive molecule is selected from the group consisting of conjugated polypeptides, cationic peptides, dextran, polyamines and chitosan.

9. The method of claim 5, wherein the angioplastic device is a stent.

10. The method of claim 9, wherein the angioplastic device has a metallic surface.

11. The method of claim 10, wherein the metallic surface is selected from the group consisting of stainless steel, gold, tantalum, nickel and titanium or any alloy thereof.

12. The method of claim 5, further comprising before the step of applying a charge to the angioplastic device, a step of washing the angioplastic device with a solvent for removing impurities at the surface of said angioplastic device.

13. The method of claim 5, further comprising after the step of applying a charge to the

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angioplastic device, a step of rinsing the angioplastic device for removing free molecules at the surface of said angioplastic device.

14. An angioplastic device for preventing restenosis in a coronary and/or peripheral artery, said device containing only a charged molecule deposited on its surface.

15. The angioplastic device of claim 14, wherein the angioplastic device is a stent or a microcatheter wire.

16. A method for depositing a radioactive charged molecule on an angioplastic device, said method consisting of the step of contacting the angioplastic device with a solution containing the radioactive charged molecule under suitable conditions for deposition of the radioactive charged molecule on the angioplastic device.

17. The method of claim 16, wherein the deposition is a passive deposition.

18. The method of claim 17, wherein the angioplastic device has gold on its surface, and wherein the radioactive charged molecule comprises a thiol-containing group for attaching to the gold on the angioplastic device.

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19. The method of claim 16, wherein the deposition is an electrodeposition.

20. A method for electrodepositing a radioactive charged molecule on an angioplastic device, said method consisting essentially of the step of applying a charge to said angioplastic device for depositing the radioactive charged molecule, said charged molecule having a charge opposite to the charge of the angioplastic device thereby electrodepositing the charged molecule on the angioplastic device.

21. The method of claim 20, wherein the charge of the angioplastic device is positive.

22. The method of claim 20, wherein the radioactive molecule comprises a β -emitter.

23. The method of claim 22, wherein the β -emitter is Antimony-124, Cesium-134, Cesium-137, Calcium-45, Calcium-47, Cerium 141, Chlorine-36, Cobalt-60, Europium-152, Gold-198, Hafnium-181, Holmium-166, Iodine-131, Iridium-192, Iron-59, Lutetium-177, Mercury-203, Neodymium-147, Nickel-63, Phosphorus-32, Phosphorus-33, Rhenium-186, Rhodium-106, Rubidium-86, Ruthenium-106, Samarium-153, Scandium-46, Silver-110m, Strontium-89, Strontium-90, Sulfur-35, Technetium-99, Terbium-160, Thulium-170, Tungsten-188, Yttrium-90 and Xenon-133.

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24. The method of claim 21, wherein the radioactive molecule is selected from the group consisting of a radioactive DNA or an analog thereof, a radioactive RNA, a radioactive nucleotide, a radioactive oligonucleotide, radioactive H_3PO_4 , radioactive diethylenetriaminepentaacetic acid, and a radioactive polyanionic complex.

25. The method of claim 24, wherein the radioactive molecule is a radioactive oligonucleotide.

26. The method of claim 25, wherein the oligonucleotide is a 8- to 35-mer oligonucleotide.

27. The method of claim 25, wherein the oligonucleotide is a 8- to 20-mer oligonucleotide.

28. The method of claim 25, wherein the oligonucleotide is a 15-mer oligonucleotide.

29. The method of claim 20, wherein the charge of the angioplastic device is negative.

30. The method of claim 29, wherein the radioactive molecule is selected from the group consisting of radioactive conjugated polypeptides, radioactive cationic peptides, radioactive dextran, radioactive polyamines and radioactive chitosan.

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31. The method of claim 20, wherein the angioplastic device is a stent.

32. The method of claim 31, wherein the angioplastic device has a metallic surface.

33. The method of claim 32, wherein the metallic surface is selected from the group consisting of stainless steel, gold, tantalum, nickel and titanium or any alloy thereof.

34. The method of claim 20, further comprising before the step of applying a charge to the angioplastic device, a step of washing the angioplastic device for removing impurities at the surface of said angioplastic device.

35. The method of claim 34, wherein the angioplastic device is cleaned with a solvent.

36. The method of claim 20, further comprising after the step of applying a charge to the angioplastic device, a step of rinsing the angioplastic device for removing free radioactive molecules at the surface of said angioplastic device.

37. An angioplastic device for preventing restenosis in a coronary and/or peripheral artery, said device containing only a radioactive charged molecule deposited on its surface.

38. The angioplastic device of claim 37, wherein the radioactive molecule comprises a β -emitter.

39. The angioplastic device of claim 38, wherein the β -emitter is selected from the group consisting of Antimony-124, Cesium-134, Cesium-137, Calcium-45, Calcium-47, Cerium 141, Chlorine-36, Cobalt-60, Europium-152, Gold-198, Hafnium-181, Holmium-166, Iodine-131, Iridium-192, Iron-59, Lutetium-177, Mercury-203, Neodymium-147, Nickel-63, Phosphorus-32, Phosphorus-33, Rhenium-186, Rhodium-106, Rubidium-86, Ruthenium-106, Samarium-153, Scandium-46, Silver-110m, Strontium-89, Strontium-90, Sulfur-35, Technetium-99, Terbium-160, Thulium-170, Tungsten-188, Yttrium-90 and Xenon-133.

40. The angioplastic device of claim 37, wherein the radioactive molecule is selected from the group consisting of a radioactive DNA or an analog thereof, a radioactive RNA, a radioactive nucleotide, a radioactive oligonucleotide, radioactive H_3PO_4 , radioactive diethylenetriaminepentaacetic acid, and a radioactive polyanionic complex.

41. The angioplastic device of claim 37, wherein the radioactive molecule is a radioactive oligonucleotide.

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42. The angioplastic device of claim 41, wherein the oligonucleotide is a 10- to 30-mer oligonucleotide.

43. The angioplastic device of claim 41, wherein the oligonucleotide is a 8- to 20-mer oligonucleotide.

44. The angioplastic device of claim 41, wherein the oligonucleotide is a 15-mer oligonucleotide.

45. The angioplastic device of claim 37, wherein the radioactive molecule is selected from the group consisting of radioactive conjugated polypeptides, radioactive cationic peptides, radioactive dextran, radioactive polyamines and radioactive chitosan.

46. The angioplastic device of claim 37, wherein the angioplastic device is a stent or a microcatheter wire.

47. A method for preventing restenosis in a coronary and/or peripheral artery comprising implanting an angioplastic device as defined in claim 37 at a site of potential restenosis in a coronary and/or peripheral artery of a patient in need of such a treatment.

48. The method of claim 20, wherein before the step of applying a charge to the angioplastic

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device, the surface of the angioplastic device is functionnalized for molecule coating.

49. The method of claim 48, wherein the angioplastic device is functionnalized with a diazonium treatment.

50. Use of an angioplastic device as defined in claim 14, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 for preventing restenosis in a coronary and/or peripheral artery.

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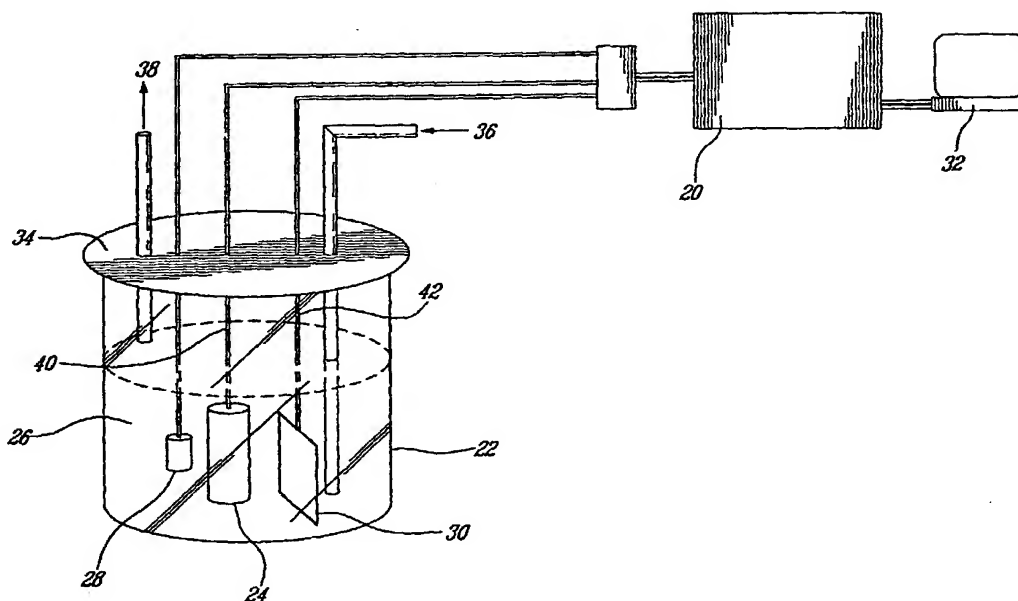
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[Continued on next page]

(54) Title: RADIOACTIVELY COATED DEVICE AND METHOD OF MAKING SAME FOR PREVENTING RESTENOSIS



(57) Abstract: The present invention relates to a rapid and reproducible electrochemical method leading to the production of radioactive angioplastic device such as stents, based on rapid and effective deposition or electrodeposition of charged radioactively coated molecule on oppositely charged surfaces (stainless or gold).

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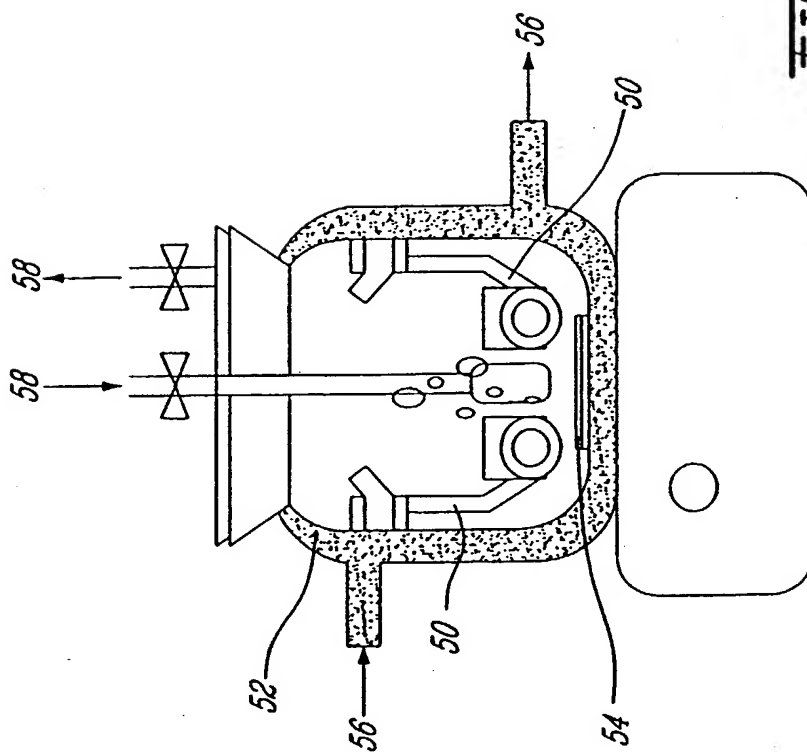


FIG. 2

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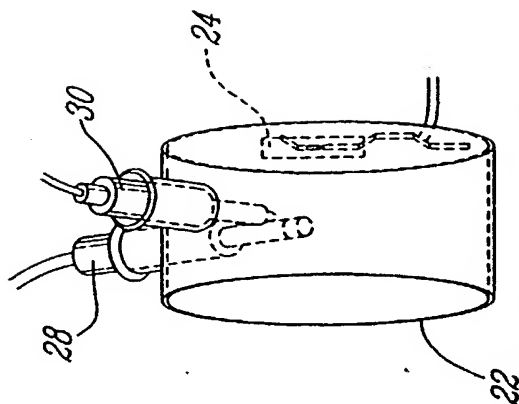


FIG. 3B

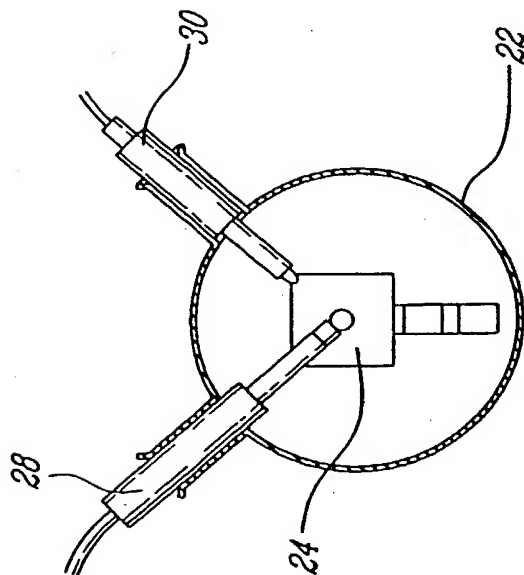
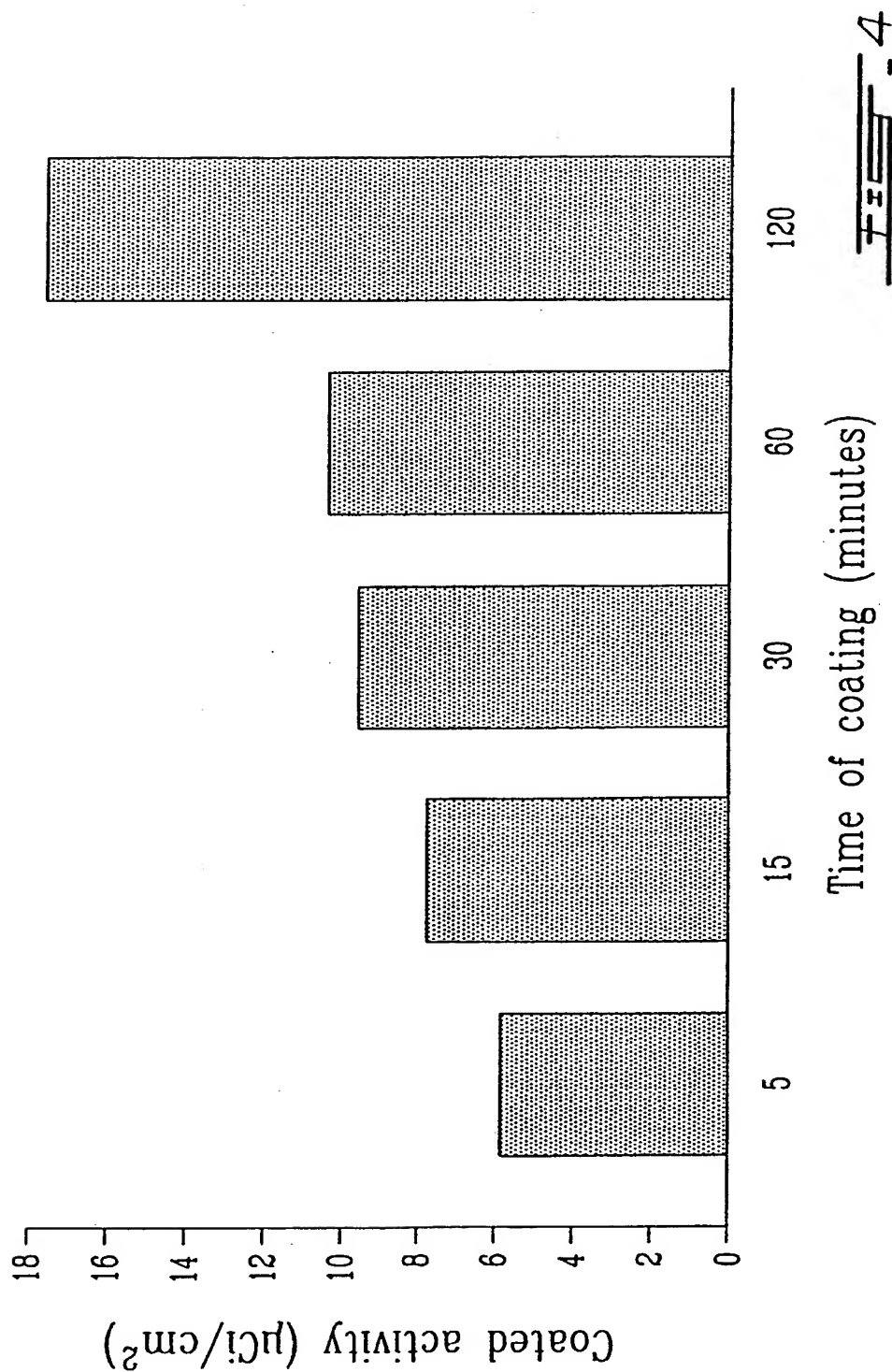
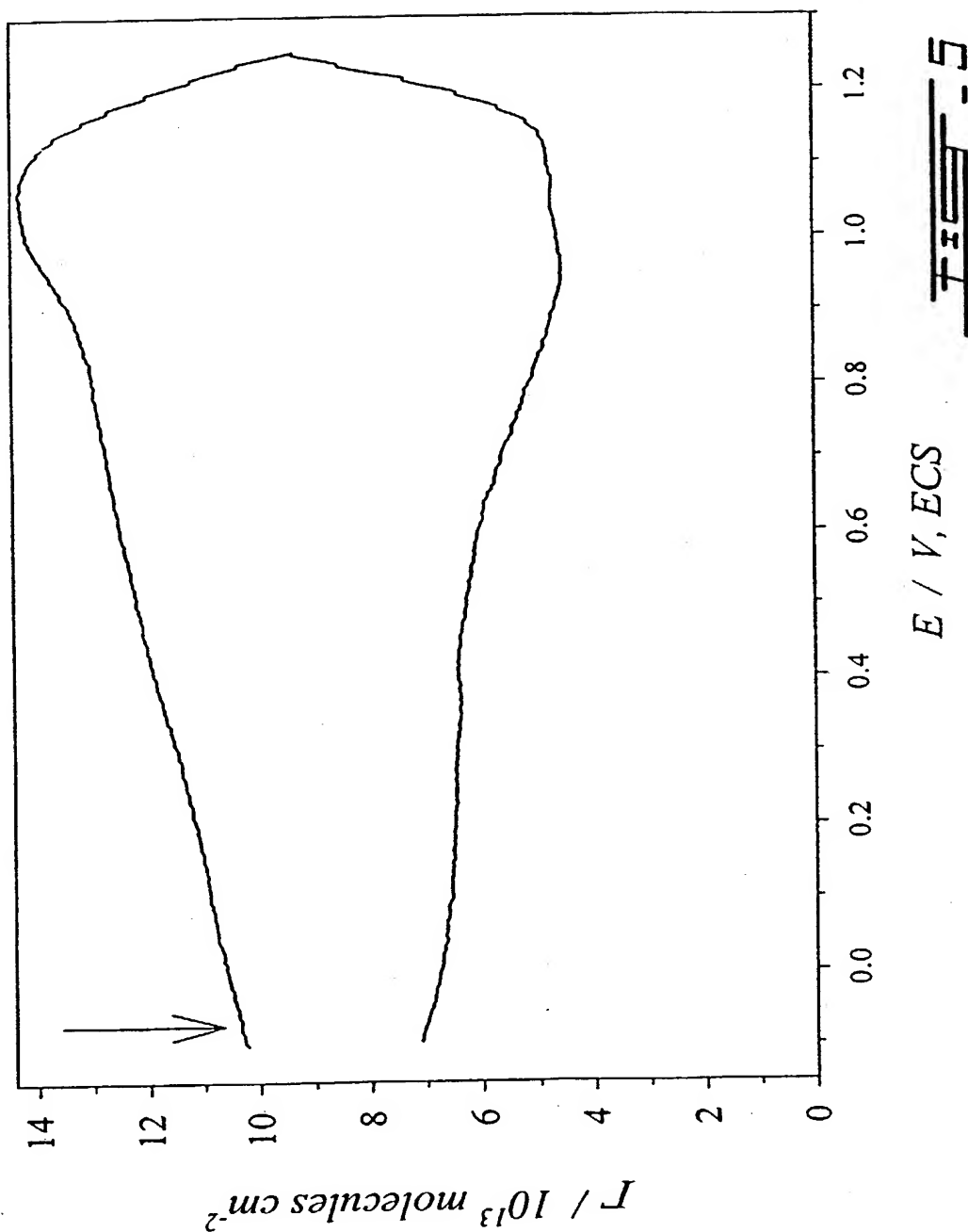


FIG. 3A

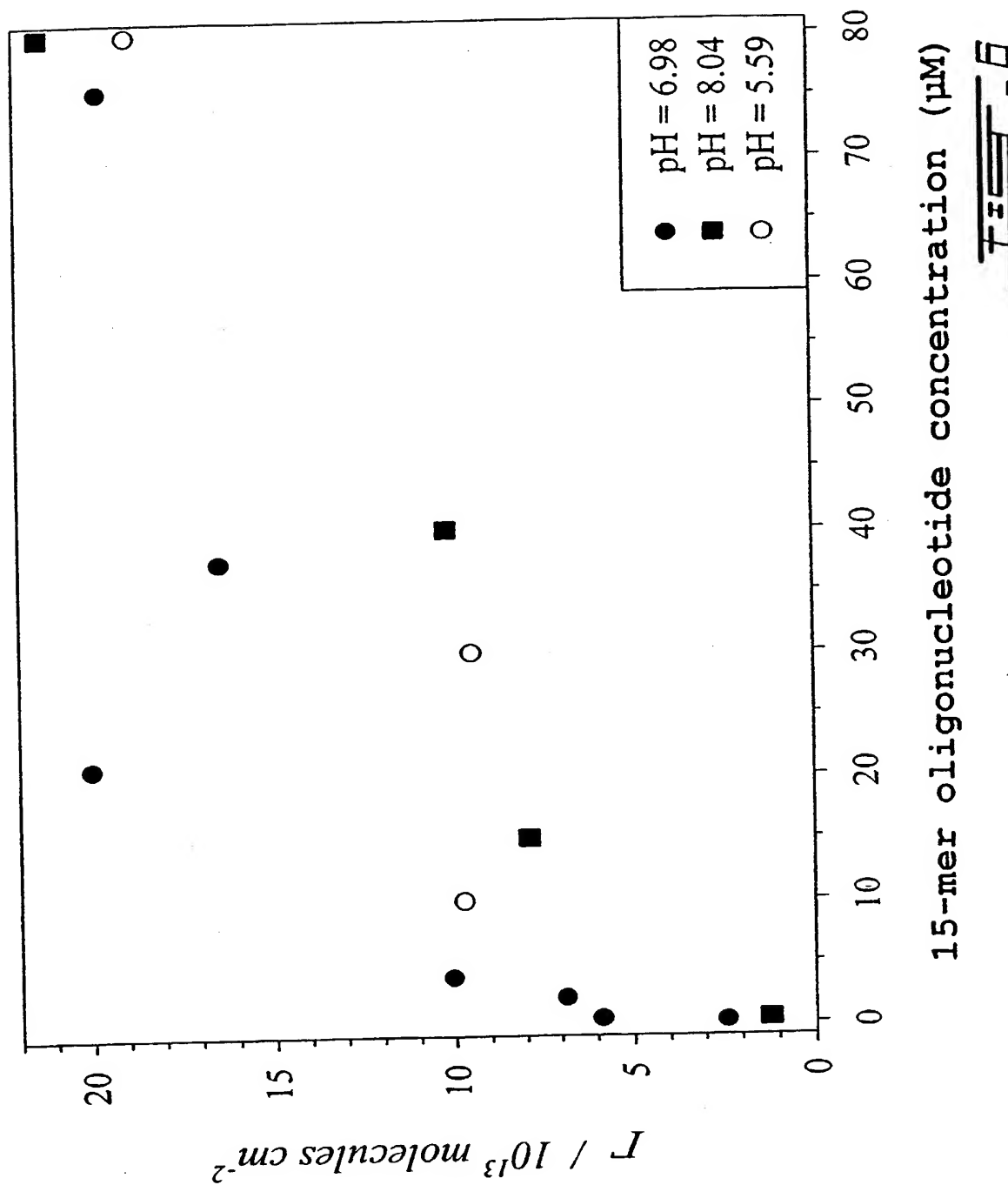
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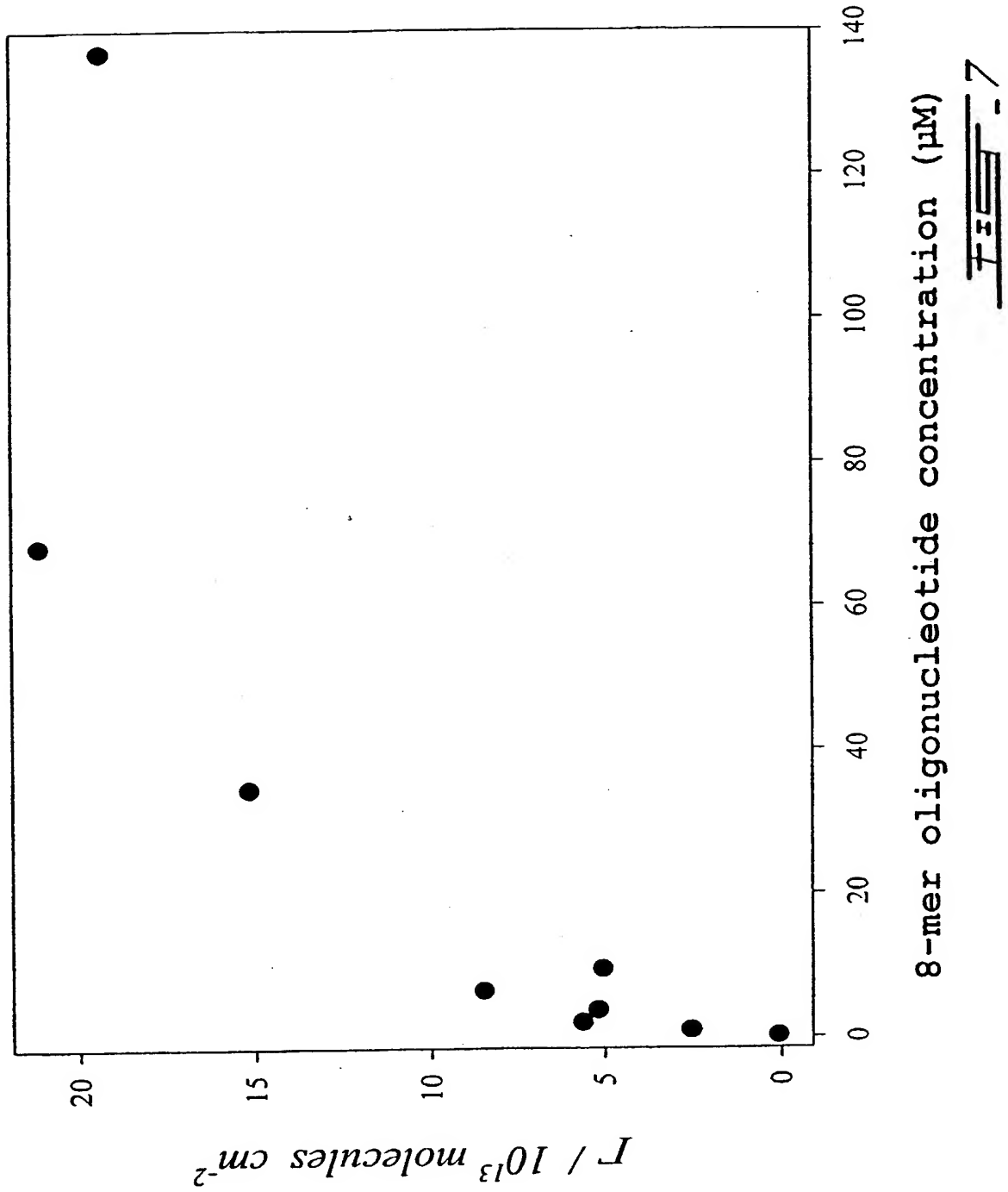
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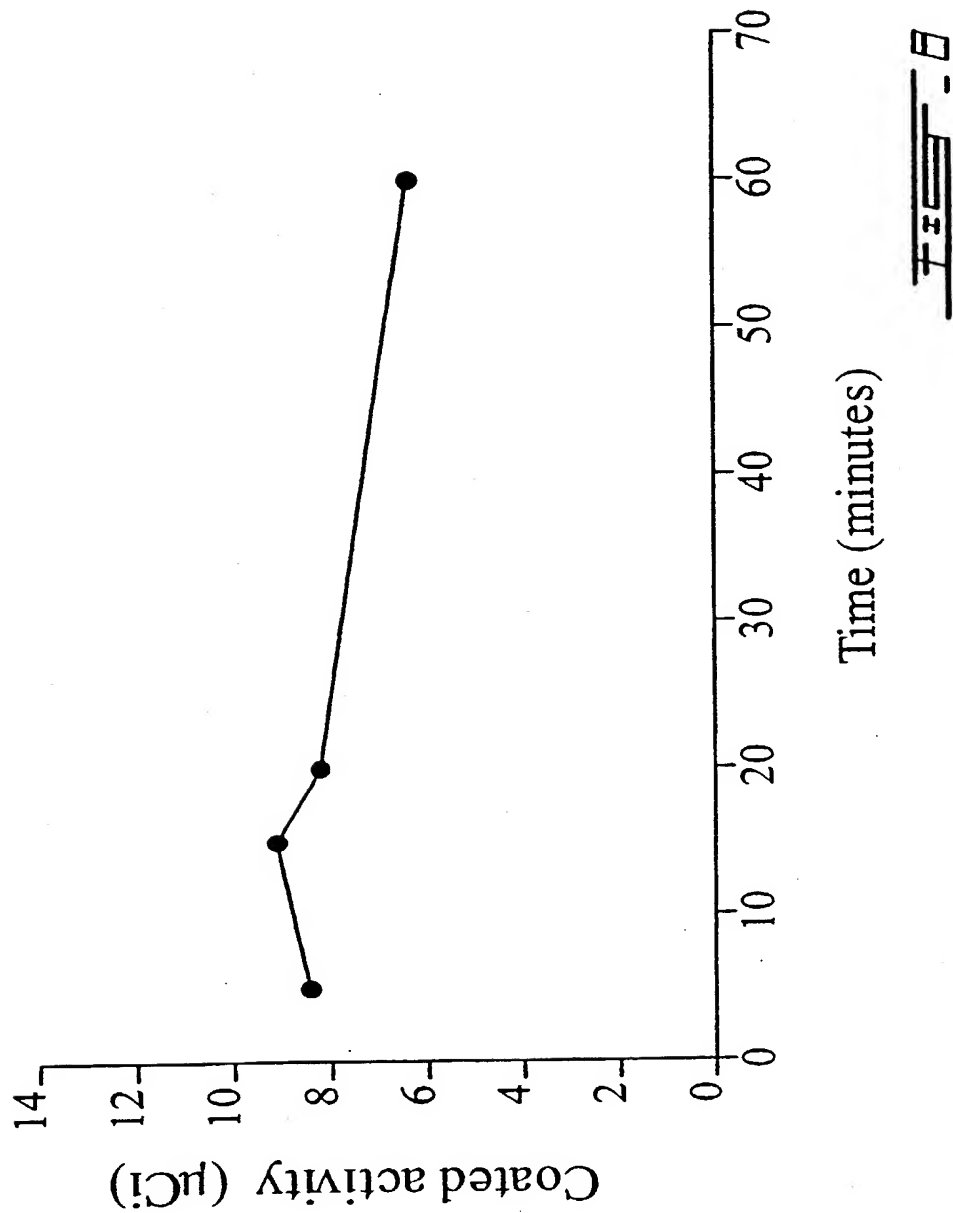
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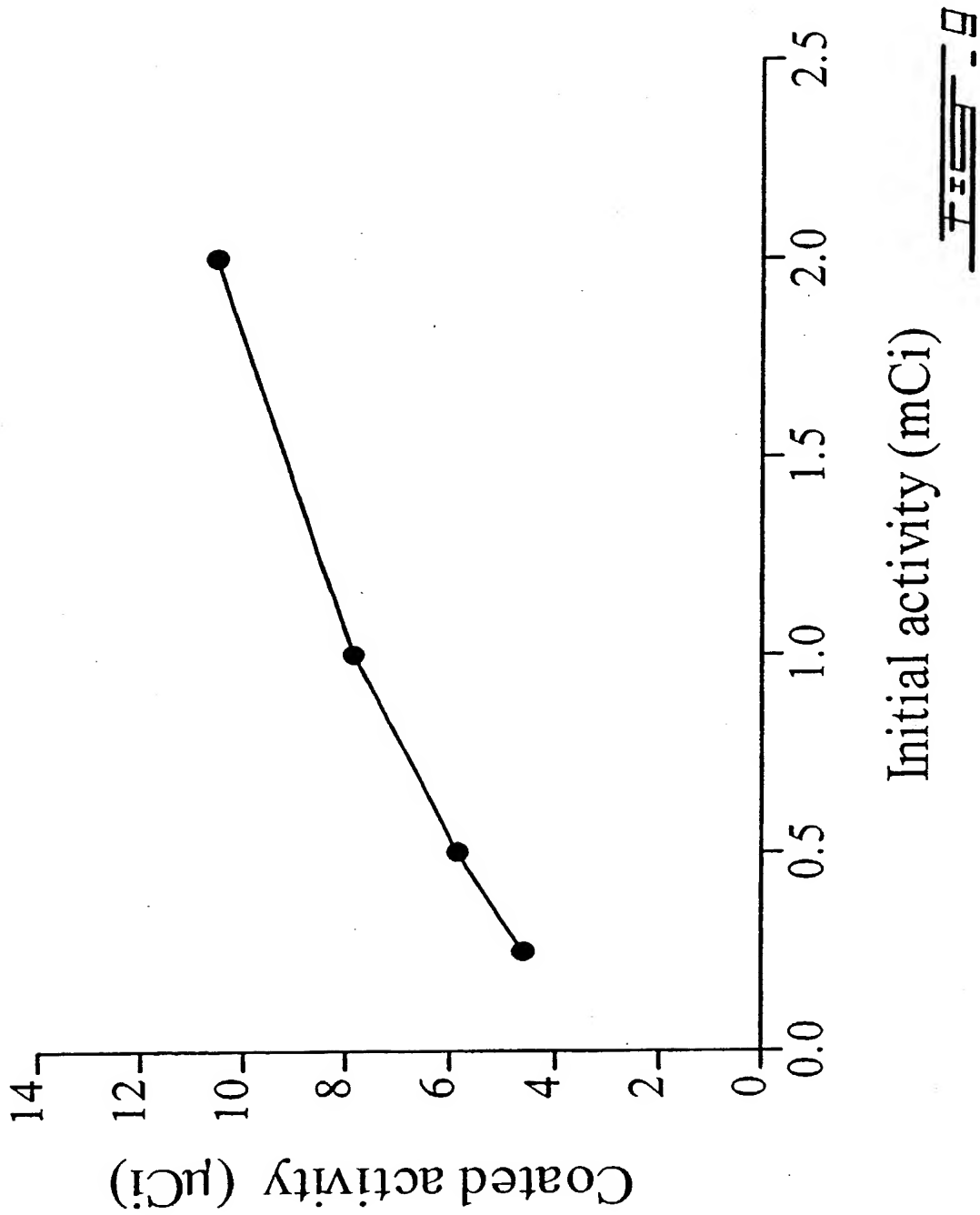
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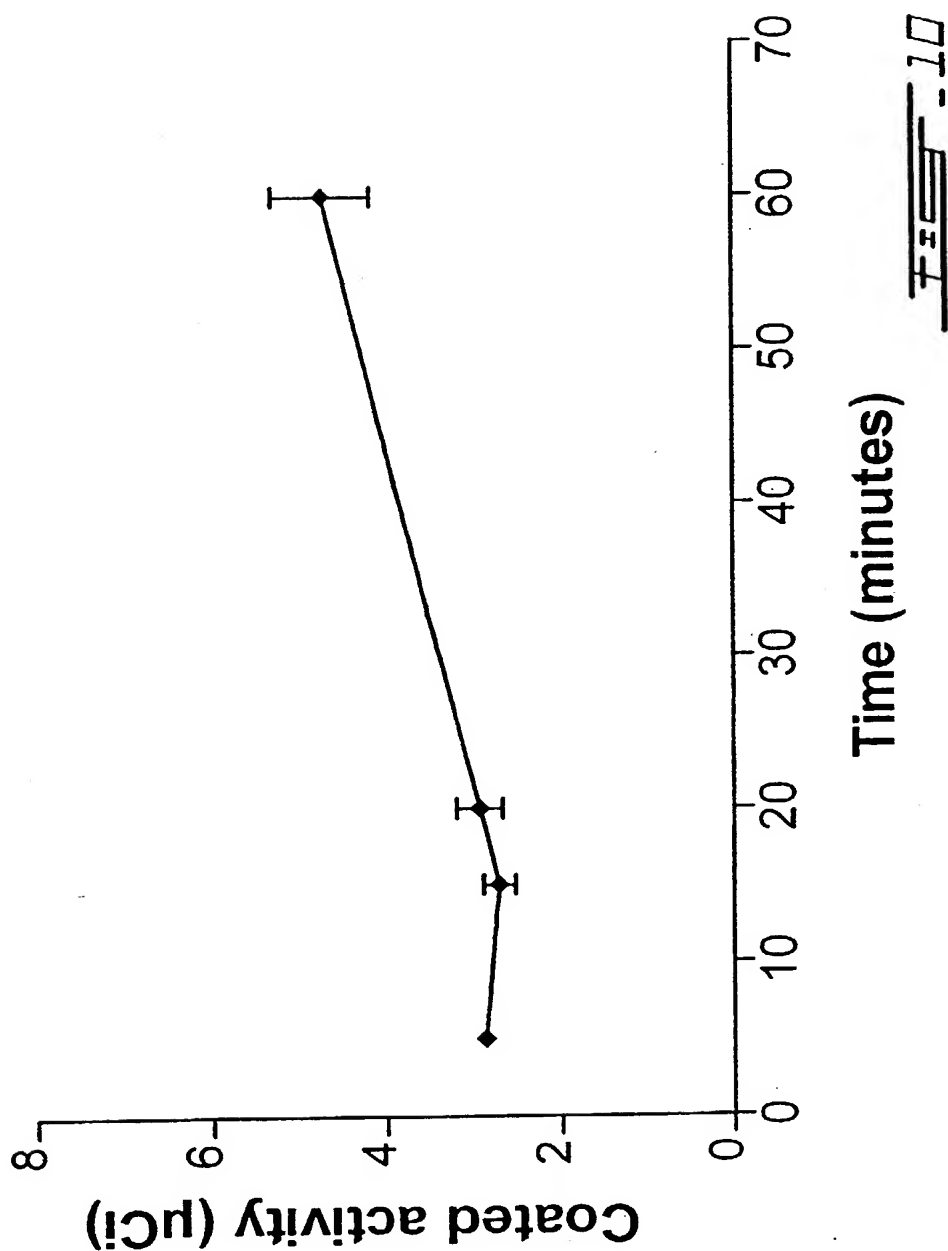
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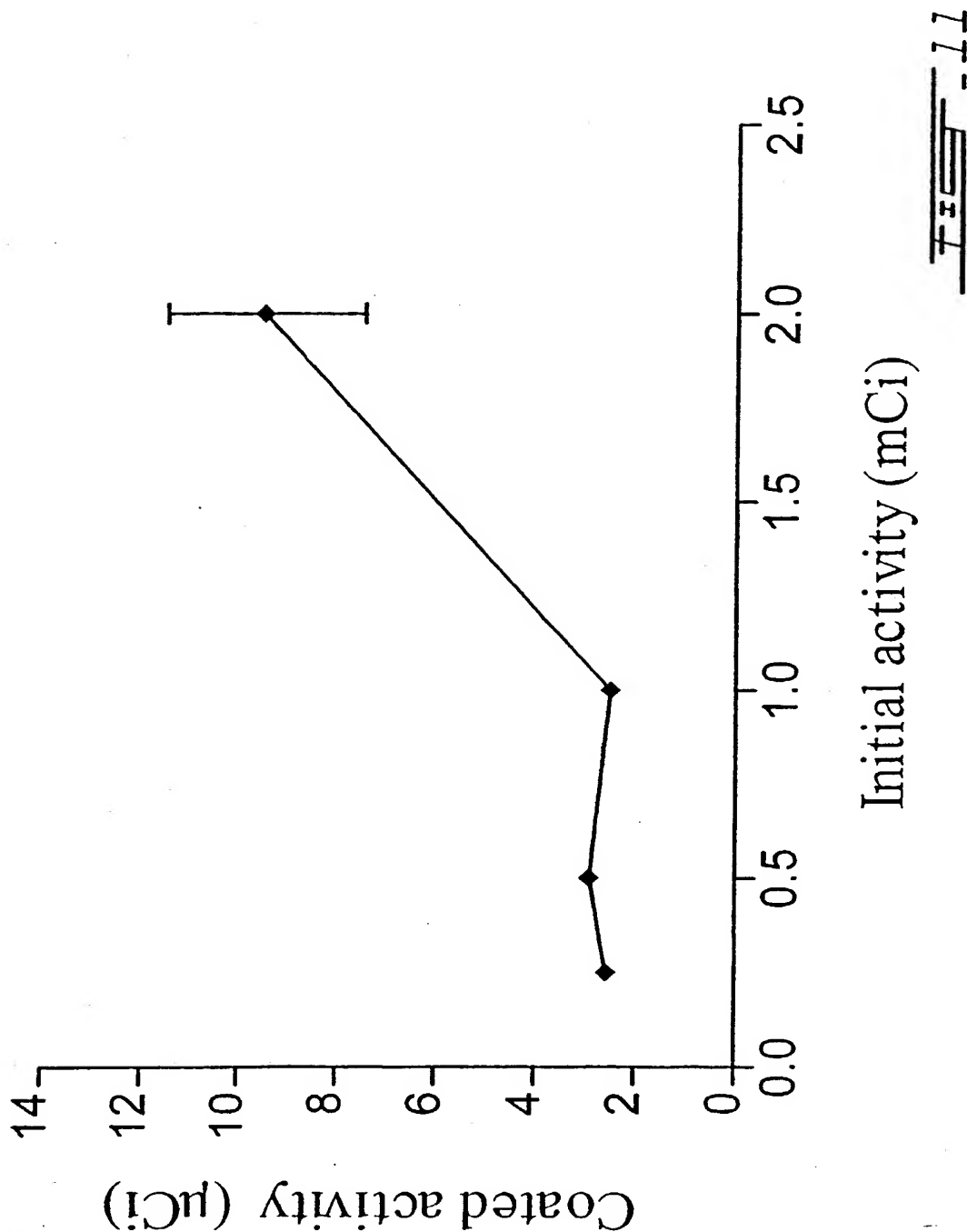
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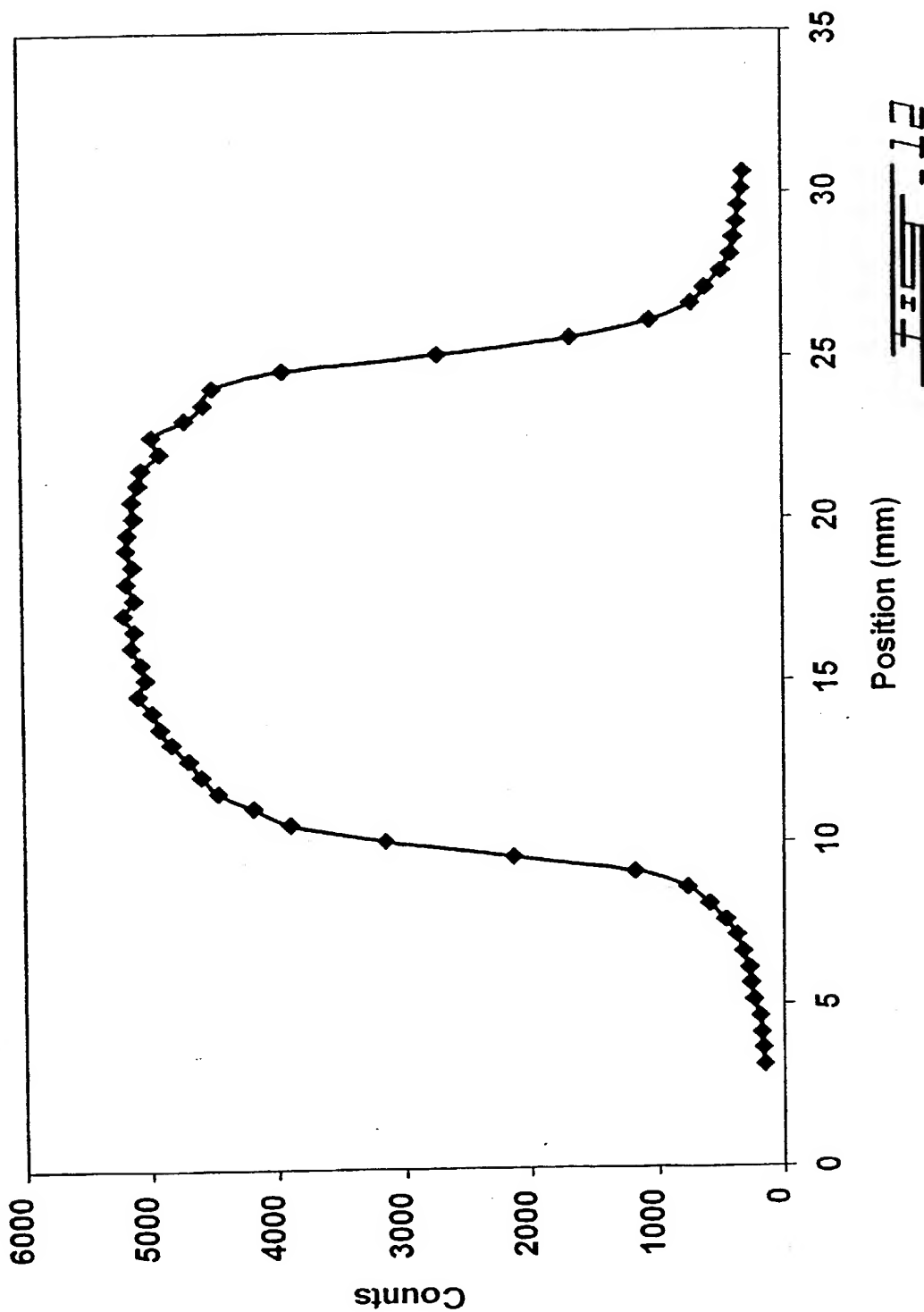


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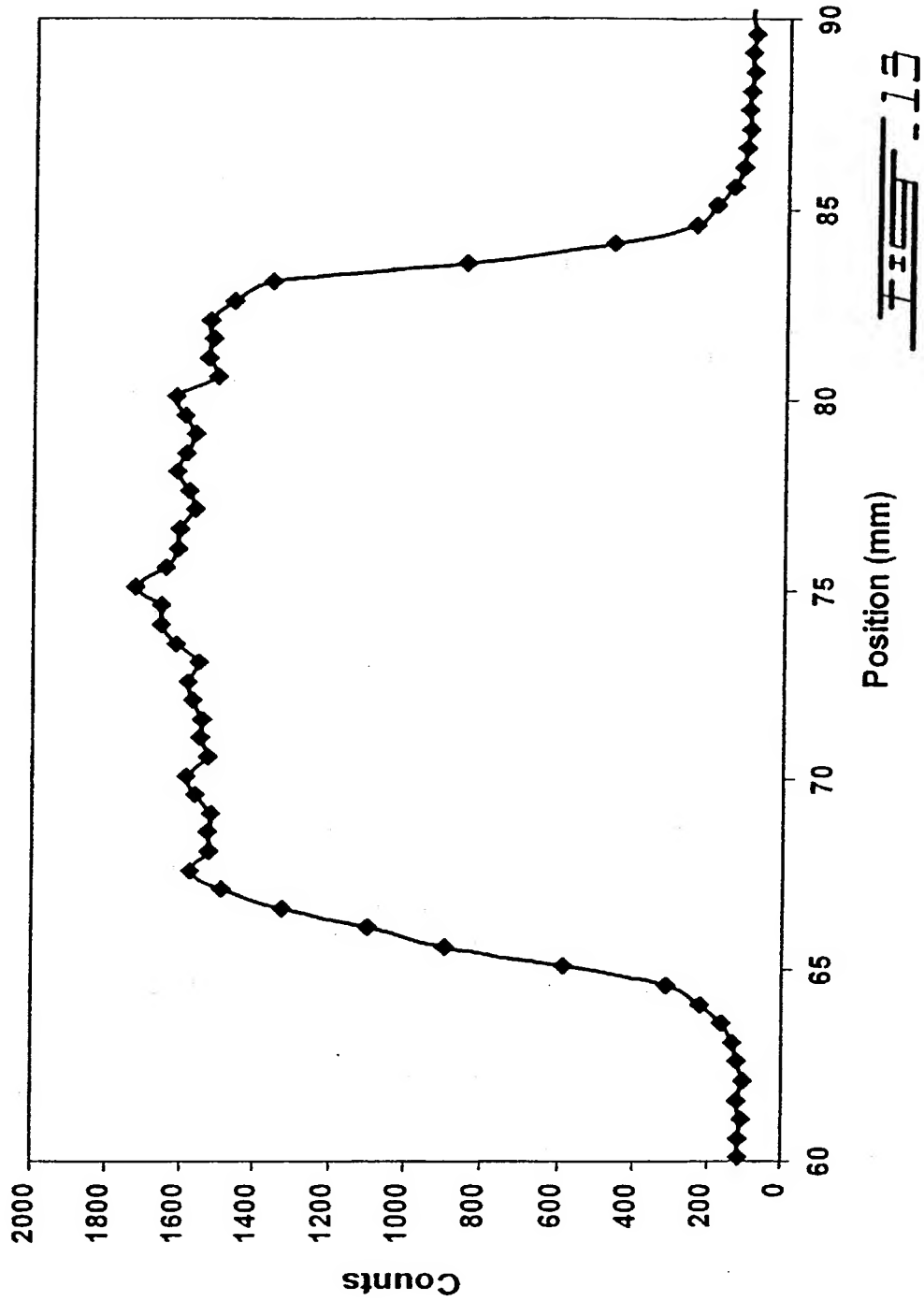
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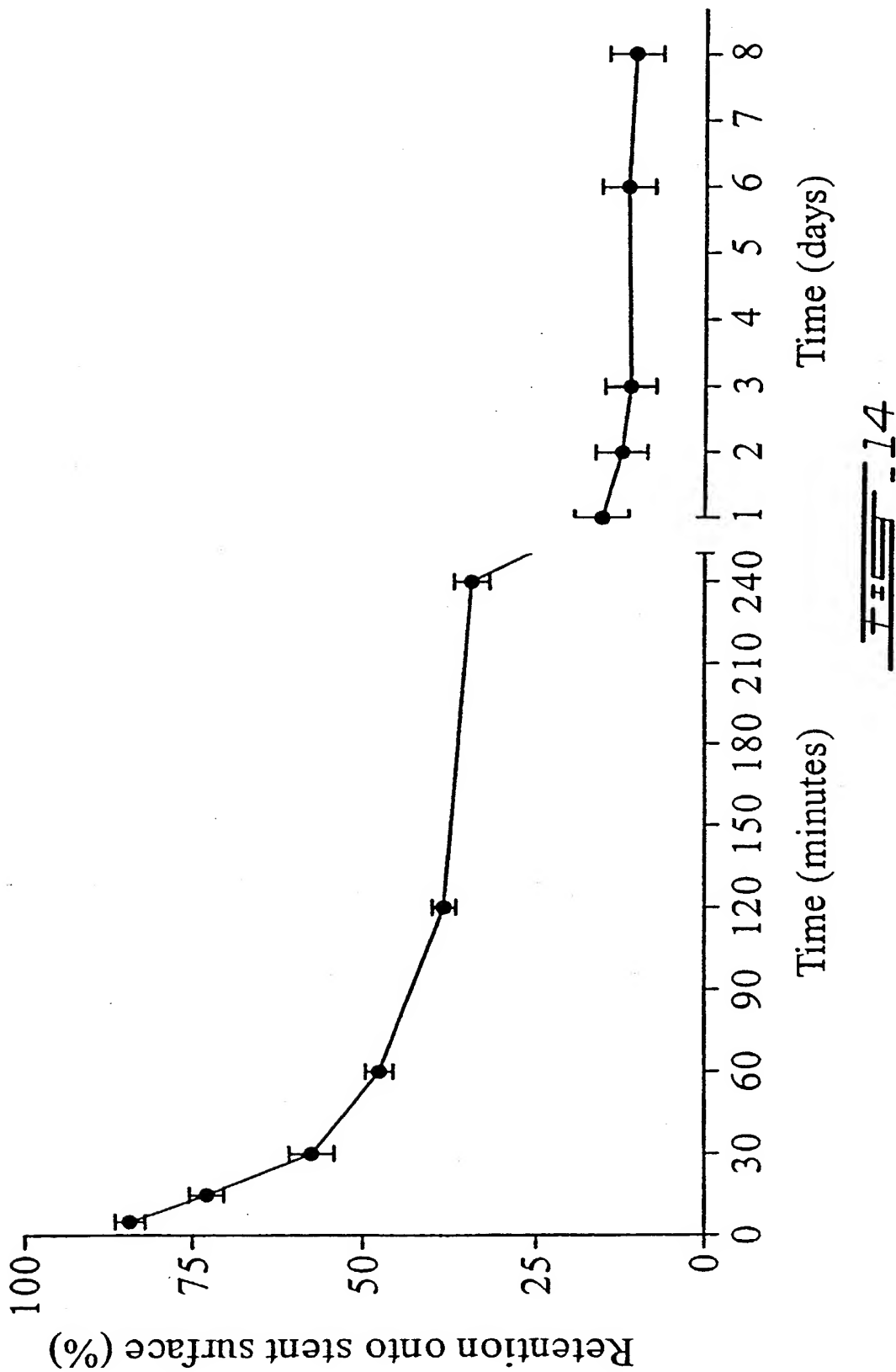
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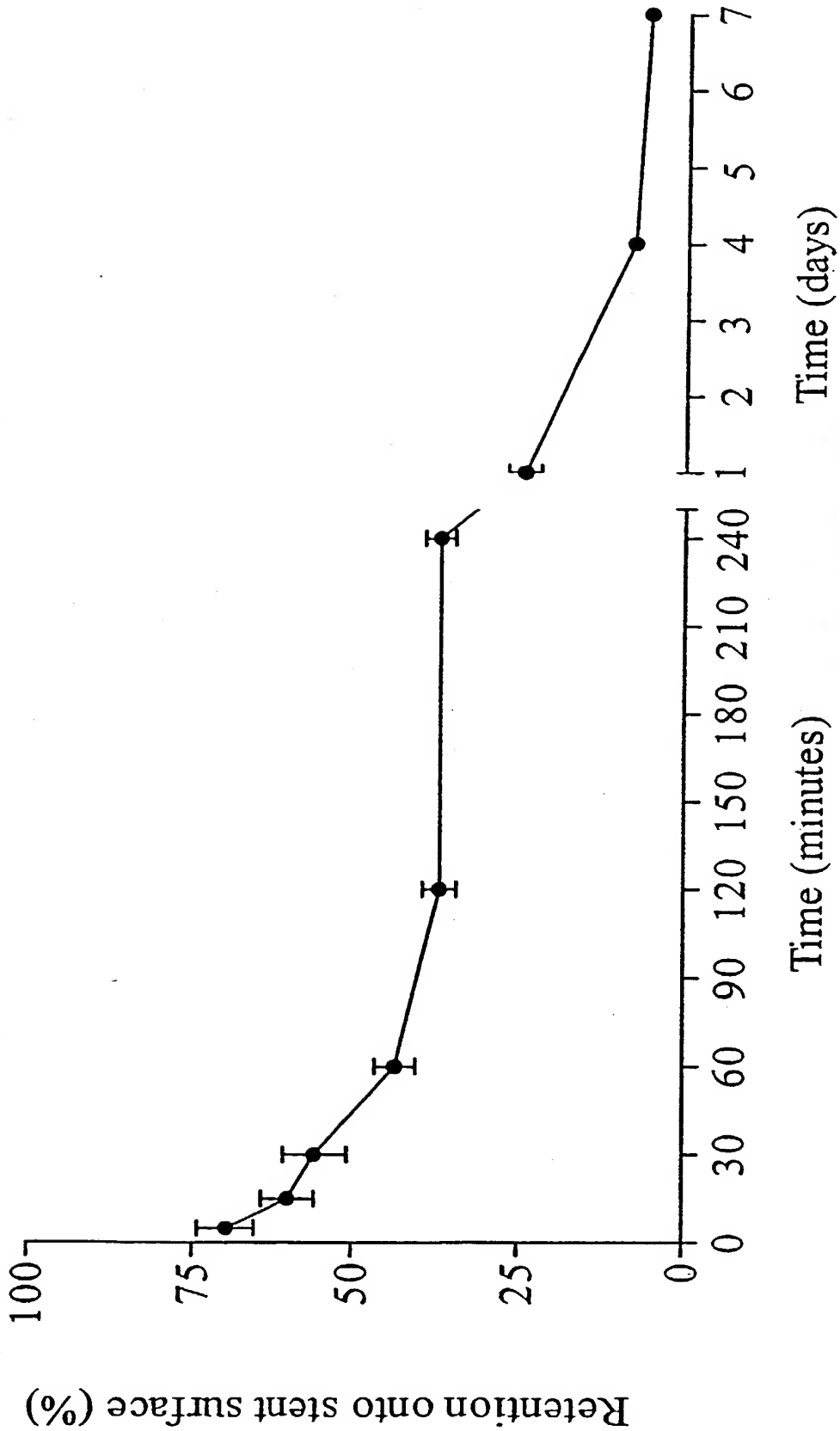


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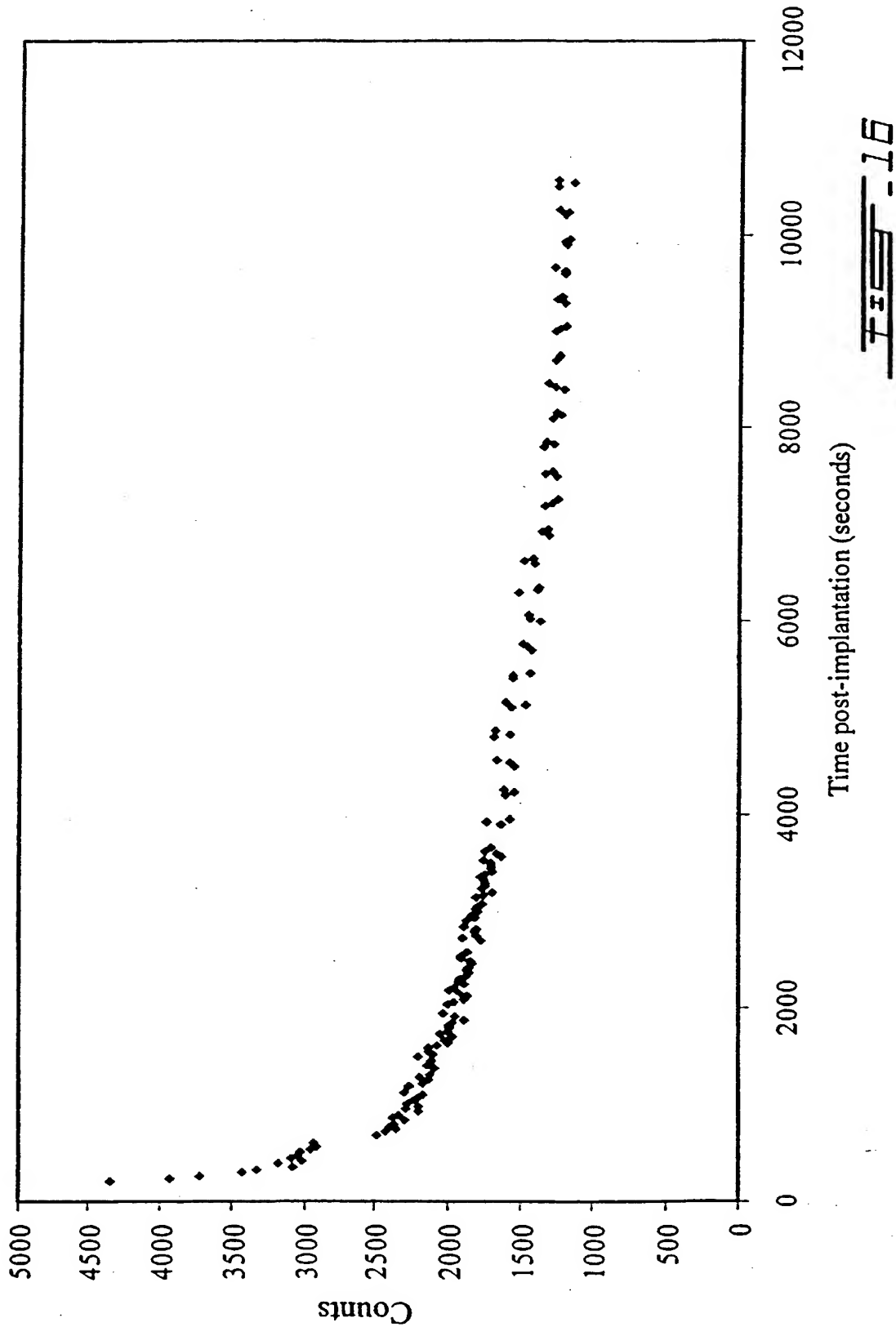
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~~FIG. 15~~

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Attorney Docket No. 24900-501 NATL

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter (an original, first and joint inventor) which is claimed and for which a utility patent is sought on the invention entitled:

**RADIOACTIVELY COATED DEVICE AND METHOD OF MAKING SAME FOR
PREVENTING RESTENOSIS**

the specification of which:

☒ was filed on February 22, 2002, as United States non-provisional application U.S.S.N. 10/069,210, based on International Application No. PCT/CA00/00974, International Filing Date August 22, 2002, bearing Attorney Docket No. 24900-501 NATL.

☐ is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

☒ I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

| Appln. Number | Country (if PCT, so indicate) | Filing Date (dd/mm/yy) | Priority Claimed | |
|------------------|----------------------------------|---------------------------|-------------------------------------|--------------------------|
| | | | Yes | No |
| CA00/00974 | PCT | 23 August 1999 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
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- ☐ I hereby claim the benefit under Title 35, United States Code, § 119(e) or §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

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I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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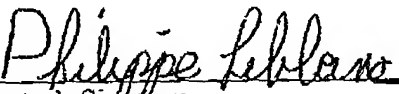
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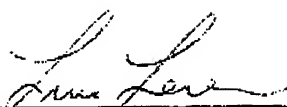
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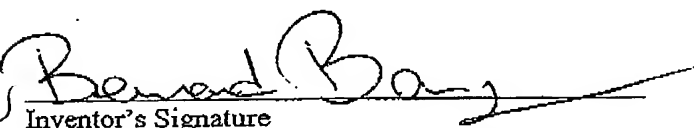
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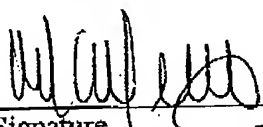
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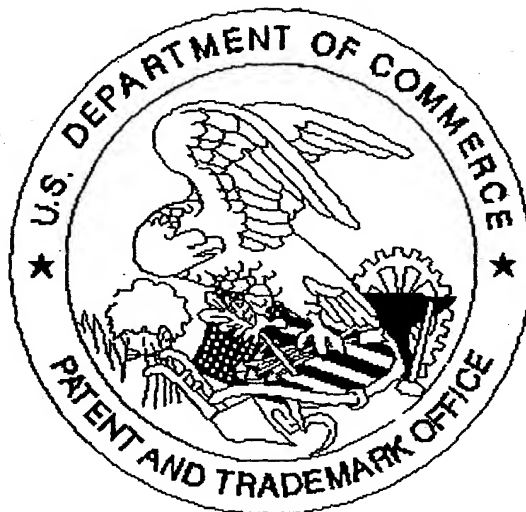
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